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THE UNIVERSITY OF ALBERTA

THE EFFECT OF HEMORRHAGIC SHOCK ON BACTERIAL TRANSLOCATION IN
A SWINE MODEL

GARY A. J. GELFAND

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

IN

EXPERIMENTAL SURGERY

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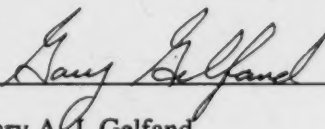
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Gary A. J. Gelfand

6603 - 102A Avenue

Edmonton, Alberta

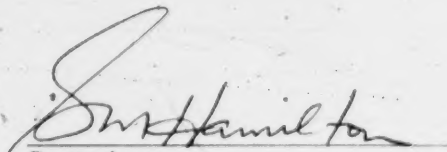
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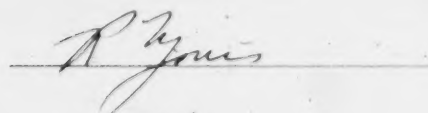
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled The Effect of Hemorrhagic Shock on Bacterial Translocation in a Swine Model submitted by Gary A.J. Gelfand in partial fulfilment of the requirements for the degree of Master of Science in Experimental Surgery


Supervisor







Date: September 7, 1989

To my mother and father.....

For their love and encouragement

To my wife Jamie.....

For whom I do everything.

GAJG

Abstract

Bacterial translocation from the gastrointestinal tract has been heralded as an explanation for sepsis associated with hemorrhagic shock. Though increased rates of translocation after hemorrhage have been shown in rodent models, close clinical correlation is lacking. To evaluate the significance of these findings, this study attempted to document these events in a more relevant model, the pig.

Twenty male swine, between 29 and 44 kilograms, were anaesthetized and the femoral artery and vein cannulated. A laparotomy was performed, and a mesenteric lymphatic vessel, the bladder, and the portal vein cannulated. An electromagnetic flow probe was placed around the craniomesenteric artery and the abdomen closed. Animals were randomly assigned to control (n=10) or experimental (n=10) groups. Experimental animals were bled 40% of blood volume. Only maintenance fluids were given. Over the next 6 hours, aerobic and anaerobic cultures of portal blood and mesenteric lymph were taken at regular intervals. Prior to sacrifice, final cultures were taken from lymph and portal blood, and from systemic blood. Mesenteric lymph nodes were retrieved and cultured.

Significant shock occurred in experimental animals as demonstrated by changes in heart rate, mean arterial pressure, urine output and other hemodynamic and biochemical parameters. Cultures of portal and systemic blood, mesenteric lymph and lymph nodes showed no significant difference ($p < 0.05$) between control and experimental animals.

In an unresuscitated swine model, this data, in contrast to that found in rodents, fails to demonstrate increased bacterial translocation within 6 hours of hemorrhage.

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This thesis represents my first foray into medical research and has provided me with valuable experience. I would not have been able to complete this project without the assistance of a number of people whom I would like to acknowledge at this time.

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List of Abbreviations

ACTH	Adrenocorticotrophic hormone
ADH	Antidiuretic hormone
ATG	Antithymocyte globulin
ATP	Adenosine triphosphate
bpm	Beats per minute
b/min	Breaths per minute
°C	Degrees celsius
C _a O ₂	Arterial oxygen content
C _{a-v} O ₂	Arterial-mixed venous oxygen content difference
C _v O ₂	Venous oxygen content
CAF	Craniomesenteric arterial flow
cAMP	Cyclic adenosine monophosphate
CI	Cardiac index
cm	Centimeter
CNS	Coagulase negative <i>Staphylococcus</i>
CO	Cardiac output
dl	Deciliter
<i>E. coli</i>	<i>Escherichia coli</i>
<i>E. aerogenes</i>	<i>Enterobacter aerogenes</i>
F _i O ₂	Fraction inspired oxygen
<i>Fuso</i>	<i>Fusobacterium</i> sp.
g	Gram
GALT	Gut-associated lymphoid tissue
[H ⁺]	Hydrogen ion concentration
HCO ₃	Bicarbonate

Hct	Hematocrit
Hg	Mercury
Hg	Hemoglobin
hr	Hour
HR	Heart rate
IV	Intravenous
Kg	Kilogram
L	Liter
LF	Lymph flow
MAP	Mean arterial pressure
MDF	Myocardial depressant factor
<i>Micro</i>	<i>Micrococcus</i> sp.
min	Minute
mg	Milligram
mm	Millimeter
mmol	Millimole
nmol	Nanomole
O ₂	Oxygen
PADP	Pulmonary artery diastolic pressure
P _a O ₂	Arterial oxygen partial pressure
P _a CO ₂	Arterial carbon dioxide partial pressure
P _v O ₂	Mixed venous oxygen partial pressure
PWP	Pulmonary wedge pressure
RAP	Right atrial pressure
RR	Respiratory rate
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
S _a O ₂	Arterial oxygen saturation

<i>S. bovis</i>	<i>Streptococcus bovis</i>
S-IgA	Secretory immunoglobulin A
SPF	Specific-pathogen-free
Std. Dev.	Standard deviation
<i>S. viridans</i>	<i>Streptococcus viridans</i>
S _v O ₂	Venous oxygen saturation
Temp	Temperature
TP	Total protein
TPN	Total parenteral nutrition
U/O	Urine output
Wt	Weight

INTRODUCTION

Shock is a common clinical entity. Though poorly understood, the term has been in use since the 1700's. Gross, in 1872, described it as a "manifestation of a rude unhinging of the machinery of life."¹ A more accurate definition of shock is "a condition in which circulation fails to meet the nutritional needs of the cell and fails to remove metabolic wastes."² With improved understanding of the pathophysiology involved, shock has been divided into four categories: hemorrhagic (or hypovolemic), cardiogenic, distributive and obstructive. Of these, hemorrhagic shock is the most common and of special interest to the surgeon.

Of all types of shock, that due to blood loss is probably the most studied. Even so, our understanding of the basic processes involved is incomplete, a point emphasized by the fact that it remains a major cause of morbidity and mortality.

Of the many unanswered questions concerning hemorrhagic shock, this thesis will examine its association with sepsis. Specifically, the role of the gut in hemorrhagic shock as a source of infection will be considered. Sepsis is a common complication of hemorrhage³, though the source is often unknown. One possible site of infection may be the gut, through a process termed bacterial translocation⁴. Of particular interest, is the possible role the lymphatics play in this process, given the extensive lymphatic drainage of the gut. Before addressing these questions, a brief review of the pathophysiology of shock is necessary.

Pathophysiology of Shock

This section will examine the pathophysiology of hemorrhagic shock. It is not intended as an exhaustive review of the subject, and the interested reader is referred to several excellent articles^{5,6,7}. Rather, those points pertinent to a discussion on the relationship between hemorrhagic shock, sepsis and the gastrointestinal tract will be emphasized. Homeostatic mechanisms will be discussed first. An overview of the changes which occur at the organ level, particularly the gastrointestinal tract, will follow. Finally, changes that occur at the cellular level will be reviewed.

I. Homeostatic Mechanisms

The cause of hemorrhagic shock is significant blood loss, and results in diminished venous return. The overall effect on the individual will depend on many factors, including rate and amount of blood loss, the patient's premorbid status and attempts at resuscitation⁶. The body responds to the insult in a variety of ways, with an overall goal of maintaining perfusion to the heart and brain⁶. It is convenient to divide these responses into two major groups, those that seek to maintain arterial blood pressure and those that regulate circulating blood volume⁵.

A. Maintenance of Blood Pressure

Though flow to vital organs determines survival, this is not easily measured and for practical purposes blood pressure is the parameter followed. Flow is related to pressure⁸ such that:

$$\text{Pressure} = \text{Flow} \times \text{Resistance}.$$

A loss of 10-20% of the blood volume will result in a decrease in mean arterial pressure^{9,10}. Hypotension is detected by baroreceptors in the carotid sinus and aortic arch¹¹. Other important stimuli, including venous pressure, volume, osmolality, pH, and pain, are integrated at the level of the hypothalamus and relayed to the autonomic nervous

system⁶. Hemorrhage causes increased sympathetic nervous system output which results in increased heart rate and myocardial contractility¹². In addition, the body attempts to maintain venous return by venoconstriction¹³. Adrenal medullary output of both epinephrine and norepinephrine is increased¹⁴.

Vasoconstriction occurs secondary to increased sympathetic tone¹⁵, with certain areas more affected than others. These include the gut, skin, and muscles, all of which have a high concentration of alpha-receptors in their vascular beds¹⁶. A host of other factors affect vascular resistance and flow. Increased blood viscosity may impair flow, as may platelet aggregation⁶. Catecholamines, especially epinephrine from the adrenal medulla, cause an increase in vascular tone, though less so in the cerebral and coronary circulations¹⁷. Angiotensin II, a potent vasoconstrictor of mesenteric and renal vessels is increased during hemorrhage¹⁸. Autoregulation, the ability to maintain blood flow in the face of decreased perfusion pressure, is an important factor in determining flow to such vital organs as the heart and brain¹⁹.

A host of vasoactive substances have been implicated in circulatory changes. Eicosanoids, arachadonic acid metabolites, are elevated in shock, and can cause vasoconstriction or vasodilation, in addition to affecting capillary permeability²⁰. Histamine has been examined as a possible mediator in shock, however, the bulk of the evidence does not support this⁶. Net effect of these different factors is to selectively shut-down blood supply to the skin, splanchnic areas, and skeletal muscle, and preserve flow to the heart and brain.

B. Restoring Blood Volume

Hemorrhage results in decreased blood volume; homeostatic mechanisms operate to restore this deficit. In addition to venoconstriction, the two most important of these are renal responses and fluid shifts.

Low pressure stretch receptors in the atria detect changes in blood volume and several mechanisms are called into play⁵. Increased renal sympathetic tone causes vasoconstriction, decreased renal blood flow¹³, and oliguria in the initial stages²¹. Neurohormonal mechanisms also affect the kidney during hemorrhagic shock. Antidiuretic hormone (ADH) is increased²¹, as is aldosterone²², with retention of sodium and water. Angiotensin causes vasoconstriction of the renal vessels, release of aldosterone, and promotes thirst²³. Atriopeptides, another group of volume regulatory hormones, play an unknown role in hemorrhage⁷.

Fluid shifts between body compartments are another important homeostatic mechanism. It is known that only 8% of the total body water is found in the vascular tree, whereas 25% lies in the interstitial space, and 67% in the intracellular space²⁴. A variety of mechanisms act to return fluid from the interstitium to the intravascular tree. As blood pressure drops, capillary hydrostatic pressure decreases and resorption of fluid from the interstitial space occurs, as one would expect from the Starling equilibrium²⁵. This process, termed transcapillary refill, has been well studied and found to return large amounts of fluid within 20 to 40 hours after moderate hemorrhage²⁶. It has been shown to have a rapid initial phase, due to decreased capillary hydrostatic pressure, and a slower second phase^{27,28}. This second phase is caused by a shift of fluid from the cell to the interstitium²⁹, which in turn, is mediated by increased extracellular osmolality³⁰. In more severe hemorrhage, interstitial water enters the cell, limiting this response³¹.

A discussion of the fluid changes which occur with hemorrhagic shock would be incomplete without addressing the role of the lymphatic system. The lymphatic system is composed of a series of collecting vessels which join and drain into the vascular tree; and the fluid found within, the lymph. The lymphatic system is known to have two major functions. The first is to transport fluid and protein from the interstitial space to the vascular tree, and the second is to transport red cells, bacteria and 'debris' from the tissue spaces to the lymph nodes³². It is the former of these which has been of most interest in

shock research, though as will be discussed later, the latter may be of more interest in considering the area of infection.

The importance of the lymphatic system in fluid balance is considerable. It has the almost unique ability to return protein from the interstitial space³³, and transports more than 100 grams per day under normal conditions³². In addition, it returns 1 to 3 litres of lymph to the intravascular volume each day³².

Investigations into the role of lymphatics in shock are not new, in fact Starling³⁴, in his original work, questioned this possibility. He rejected this idea, a decision which had a profound influence on subsequent research. Wessley³⁵, re-examined this area, and felt that the contribution of the lymphatics in hemorrhage was minimal. Cope and Litwin³⁶, in a dog model found quite the contrary, concluding that within 24 hours of hemorrhage, the lymphatic system had returned over twice as much protein as had been lost by hemorrhage. Other studies have either confirmed³⁷ or denied^{38,39} the importance of the lymphatic system in the response to hemorrhage, but have used a variety of experimental situations in different animal models. The question remains unresolved.

It is only within the last twenty years that researchers have come to realize that the lymphatic vessels themselves are not merely passive conduits, but capable of pumping fluid independently⁴⁰. In light of this, the role of the lymphatics in hemorrhage has been re-examined⁴¹. In a hemorrhage sheep model, Hayashi et al⁴² found increased pumping in mesenteric lymphatics in a hemorrhaged sheep model, up to 6 times that of controls. Thus, while the role of the lymphatic system in shock is uncertain, it will no doubt be the source of further investigations.

C. Other Homeostatic Mechanisms

A multitude of other responses to blood volume depletion exist. Metabolic alterations, the result of a variety of neurohormonal changes, are important. Adrenocorticotrophic hormone (ACTH) and cortisol are essential⁷. Glucagon, released in

large amounts during shock, contributes to hyperglycemia, increased cardiac output and vasodilation of important vascular beds⁴³. Other mediators, including thyroid and related hormones⁴⁴, endorphins and enkephalins⁴⁵, and eicosanoids²⁰, may play important roles in shock.

II. Organ Function in Shock

Perfusion, and consequently function, of different organ systems is altered in hemorrhagic shock. Flow to the heart and brain are preserved, due, at least in part, to autoregulation¹⁹. By definition, major alterations in cardiac function are not an initial finding in hemorrhagic shock. Nevertheless, diminished venous filling results in decreased cardiac output. Some investigators contend that certain mediators produced in shock, such as myocardial depressant factor (MDF), adversely affect cardiac function^{46,47}. Renal blood flow is also decreased, with blood being preferentially shunted to the juxtamedullary nephrons¹⁹. While the respiratory system is clearly affected by shock, the term 'shock lung' is a misnomer, for no clear relationship has been shown between the adult respiratory distress syndrome (ARDS), and hypotension alone⁴⁸.

A discussion of sepsis and hemorrhagic shock would be incomplete without considering the role of the immune system. Much of the work done in this area has looked at the effect of trauma on the immune system, rather than the specific effects of hemorrhage. The two basic areas to be considered are cellular- and humoral-mediated immunity.

T-cells function in cell-mediated immunity, and are adversely affected by trauma and hemorrhage. MacLean et al.⁴⁹ documented impaired delayed hypersensitivity in critically ill patients. O'Mahony et al.⁵⁰ confirmed this, and showed a decrease in the T-helper to T-suppressor cell ratio in multiple trauma patients. Abraham and Regan⁵¹ demonstrated decreased production of interleukin 2, an important mediator in T-cell function, in hemorrhaged patients.

Hemorrhage and trauma have been shown to affect humoral-mediated immunity as well. Complement activation is decreased after soft tissue injury⁵², and opsonization is decreased after major trauma⁵³. From these studies, it is clear that the immune system is adversely affected in trauma, and likely in hemorrhagic shock.

Important changes occur to the gastrointestinal tract in hemorrhagic shock. Sympathetic stimulation causes splanchnic vasoconstriction⁵⁴, though the extent to which this occurs in humans is not clear. Studies in canine shock models show intense vasoconstriction, with hepatic outflow obstruction, and gut mucosal ulceration⁵⁵. This does not appear to be the case in other experimental models or humans⁵⁶, where sympathetic vasoconstriction does not appear to be as important⁵⁷. Vasoactive mediators may be more important in splanchnic blood flow following hemorrhage⁵⁸. The net effect is that initially resistance increases, but returns to pre-hemorrhage levels after several hours of hypotension⁵⁹. This drop may be due to a decrease in neurotransmitter concentration, or autoregulation⁶⁰. This fading effect of sympathetic stimulation also affects the capacitance vessels, such that intestinal blood volume gradually increases with time in shock⁶¹, though this does not appear to be of major importance in animals other than the dog. Shock also appears to increase capillary permeability⁵⁸.

Morphological changes to the intestine occur with hemorrhage, the most common being mucosal ulceration⁵⁸. The exact cause is unknown, but may be tissue hypoxia⁶², superoxide radicals⁶³, or pancreatic proteases⁶⁴.

Researchers have attempted to implicate the gastrointestinal tract as an important determinant of survival from shock episodes⁶⁵. Possible mechanisms include fluid loss⁶⁵, endotoxins⁶⁶, or endogenous toxins⁶⁷ released by the gut during hemorrhage. Of these, fluid loss may be important in the dog, but not in other species or man⁵⁸. Work continues on characterizing possible endogenous toxins originating from the intestine.

The original suggestion of the role of endotoxin fell into disfavor after finding that germ free animals were equally susceptible to hemorrhagic shock and intestinal insult⁶⁸. As will be discussed later, there is renewed interest in this area.

III. Cellular Changes in Hemorrhagic Shock

While major advances have been made in understanding pathophysiology of shock at an organ level, equally important is the appreciation that shock is a cellular phenomenon. Studies in this area have not lead to major clinical advances yet, but hold promise for the future.

Shock profoundly alters the cell's energy supply. Adenosine triphosphate (ATP), a high-energy phosphate nucleotide and an important intermediary in cell function, is decreased in shock⁶⁹. This is due, at least in part, to a changeover to anaerobic metabolism, an inefficient form of energy production⁷⁰. Decreased levels of ATP cause a decrease in adenosine 3',5'-cyclic monophosphate (cAMP)⁷¹, which is vital in the regulation of many of the cell's processes. Anaerobic metabolism causes increased production of pyruvate, and thus lactate, resulting in acidosis⁷⁹.

Mitochondria, in the presence of oxygen, provide the bulk of the cell's energy through the tricarboxylic acid cycle and electron transport system. In shock, several significant changes occur. Metabolism, especially of alpha-ketoglutarate, is depressed⁷². In addition, the ability to increase the rate of oxidative phosphorylation in response to increased needs is impaired in hemorrhagic shock⁷². The exact cause of this dysfunction is uncertain, however structural changes⁷³, loss of cofactors⁷⁴, increased levels of calcium⁷⁵, or altered mitochondrial transport mechanisms⁷⁶, may be responsible. Whether these changes occur in mitochondria of all organs is uncertain⁷⁵.

The cell membrane is affected by shock. Early studies by Shires and his coworkers^{77,78} examined the effect of hemorrhagic shock on body fluid distribution. With severe hemorrhage, functional extracellular fluid decreased without a proportional increase

in plasma volume.^{77,78} Subsequent studies showed that interstitial fluid shifts into the cell after severe hemorrhage, and is associated with a decrease in transmembrane potential difference⁷⁹. Sodium and potassium distribution, vital in maintaining membrane potential, are altered such that intracellular potassium is decreased, and intracellular sodium increased. It appears likely that the manner in which these cations are transported is abnormal⁷⁰, though the exact mechanism is unknown. An abnormality in the Na⁺-K⁺-ATPase pump, or an alteration in cell membrane permeability are likely explanations⁷⁰.

One intracellular organelle which deserves special consideration is the lysosome. Lysosomes contain autolytic enzymes, and are found in highest concentration in the splanchnic system⁷⁰. After cell death, lysosomes are known to release their enzyme contents, and the question has been raised whether earlier disruption is part of the process leading to irreversible shock⁸⁰. Evidence confirming this is lacking at present.

The net effect of all these changes is severe cellular dysfunction. If unchecked, this will result in cell death, and ultimately, death of the organism. Considerable debate, beyond the scope of this paper, has centered around such questions as what is the final common pathway in shock⁸¹, and when do these changes become irreversible⁸².

Much has been learned in the past fifty years regarding the pathophysiology of hemorrhagic shock. Attention has turned from organ systems to cellular dysfunction, but many unanswered questions remain. These continue to fuel research into the field, as does persistent morbidity and mortality associated with hemorrhagic shock. The rest of the thesis will examine one emerging topic

Role of the Gut in Hemorrhagic Shock

Hemorrhagic shock is a major cause of morbidity and mortality. Trauma, the commonest cause of hemorrhagic shock, was responsible for 165,000 deaths in the United States in 1982⁸³. Of these, 30% occurred within several hours of injury, and were due to either hemorrhagic shock or head injuries⁸³. A further 20% died days to weeks after the

insult, and over 80% of these were due to sepsis and multiple organ failure⁸³. The aim of this section is to examine the role of the gut in sepsis associated with hemorrhagic shock. The pathophysiology has already been detailed (vide supra). The importance of sepsis in hemorrhagic shock will be outlined first. A review of the early studies in this field will then be presented, in order to appreciate why this line of investigation was abandoned for almost fifteen years. This will be followed by a review of bacterial translocation, the current theory which seeks to explain the association between hemorrhage and sepsis.

I. Sepsis in Hemorrhagic Shock

Studies of critically ill patients, have revealed that hemorrhagic shock is a major risk factor in the development of infection^{84,85,4}. Conversely, sepsis is a major source of mortality and morbidity in shock patients, and plays a critical role in development of multiple-organ-failure syndrome⁸⁶. In many instances, the site of infection is not found. Garrison and his colleagues⁸⁷, in examining Enterococcal bacteremia, found over 40% of cases had no discernible primary focus of infection. Fry and his coworkers⁸⁸, found similar results in patients with *Bacteroides* bacteremia. Stone et al.⁸⁹ demonstrated that in dogs introduction of *Candida* into the gastrointestinal tract could result in candidemia. Recently, Rush⁹⁰ examined a series of 50 patients admitted to a surgical intensive care unit. When classified according to initial blood pressure, it was found that in those with a blood pressure of less than 80 mmHg systolic, 56% demonstrated bacteremias for which no source was found. This was compared to 4% of patients with pressures of greater than 110 mmHg, and 28% of patients with pressures between 80 and 110 mm Hg. Of those with blood pressures of less than 80 mmHg, almost one half (22%) grew gram negative organisms, either alone or in combination with gram positives.

From these studies it is clear that sepsis after hemorrhagic shock is not an infrequent event. Furthermore, the source of infection in many instances is obscure. Given that the gut is one of the major sources of endogenous bacteria, it is not surprising

that current investigators turn their attention to the gastrointestinal tract. As will be seen, they were not the first to consider this possibility.

II. Early Investigations

The idea that the gastrointestinal tract, sepsis and hemorrhagic shock could all be related was first looked at over 40 years ago⁹¹. Jacob and his associates, Schweinburg and Fine, were among the earliest investigators. In a hemorrhagic shock model in dogs, they were able to significantly alter survival by pretreating the animals with various antibiotic routines⁹². Though blood cultures taken while the dogs were alive were consistently negative, those taken shortly after death, as well as cultures of liver specimens, showed substantial growth of *Clostridia*. In a later study⁹³, liver homogenate from severely shocked dogs was injected intraperitoneally into other animals who had been minimally bled. These dogs died from hemorrhage that was otherwise never fatal. Schweinberg et al.⁹⁴ also examined the ability of bacteria to cross the intestinal wall, and found that radiolabeled *Escherichia coli* were able to migrate from the gastrointestinal tract to the peritoneal cavity after prolonged chemical irritation of the peritoneal cavity.

Other researchers in the field, notably Lillehei and his associates at the University of Minnesota, also felt the gut played an important role in hemorrhagic shock⁶⁵. They discounted the theories regarding the role of the gut in sepsis largely on the basis of work in germ free animals (vide infra). Instead, they emphasized the phenomenon of 'stagnant anoxia', whereby hemorrhage ultimately led to pooling of blood in the splanchnic circulation.

Fine continued to promote his theories on the gut in sepsis and shock into the late 1960's^{95,66}, however several major criticisms were raised. First, the dog is less than an ideal model for studies in hemorrhagic shock⁸². There are several reasons for this, one of the most important being that the dog has a continuously septic splanchnic circulation⁹⁶. In addition, its splanchnic circulation is much more sensitive to vasoconstriction than is a

human's, and is prone to hepatic outflow obstruction⁵⁵. Second, others were unable to reproduce the work done by Fine and his associates, notably with regards to the effects of antibiotics on survival⁹⁷. Third, neither Zweifach⁹⁸ or Carter⁶⁶ was able to demonstrate differences between hemorrhagic shock in germ-free animals and those with normal flora. For these reasons, this line of research was abandoned for over ten years.

III. Bacterial Translocation

While considerable doubt was raised about the studies done by Fine and others, the finding of sepsis in hemorrhagic shock seemed more than coincidental. The fact that frequently no focus of infection could be identified caused researchers to re-examine the possibilities. Attention again turned to the gastrointestinal tract. It was at this time, that the phenomenon of bacterial translocation was first described.

Bacterial translocation is defined as "the passage of bacteria from one location (e.g., the gastrointestinal tract) to another (e.g., the mesenteric lymph nodes), without suggesting anything concerning the mechanisms involved."⁹⁹ The term was first coined by Berg and his coworkers⁴, though earlier groups had proposed similar theories^{100,101}. Translocation is usually documented by culturing mesenteric lymph nodes, spleen, liver or blood samples.

Though in constant contact with a wide variety of microorganisms, the gastrointestinal tract lining is remarkable in its ability to resist infection. Two major factors appear important¹⁰². The first is the mechanical and chemical barrier formed by tight intercellular junctions, mucus secreted by the epithelium, and the indigenous microflora, especially anaerobes¹⁰³. The second is the immune system.

Much of the work on bacterial translocation has necessarily been done in animal models. Early work set out to show that, under resting conditions, indigenous bacteria were unable to translocate to a significant degree. This implied that either the bacteria did not cross the mucosal barrier, were killed in transit or were killed by the reticuloendothelial

system. It should be emphasized that translocation refers to indigenous bacteria, and not to those bacteria which are either known pathogens, or nonindigenous. *Salmonella*, for example, readily penetrate the intestinal barrier¹⁰⁴. Berg and Garlington⁴, in specific-pathogen-free (SPF) mice, were unable to culture bacteria from the mesenteric lymph nodes, spleen or liver. Other researchers obtained comparable results in different species as well as humans¹⁰⁵. Dogs, as noted, are an exception to this rule. Having shown that under normal circumstances indigenous bacteria do not readily translocate, research began to identify factors which might promote translocation. Several factors have been identified and will be reviewed.

A. Intestinal Permeability

Under normal circumstances, the gastrointestinal tract is sufficiently impermeable to indigenous bacteria to prevent recurrent bacteremias^{99,105}. This function varies with age, and it has been shown in animal models that neonates are more susceptible to translocation¹⁰⁶. Experiments designed to alter intestinal permeability have demonstrated an increase in the rate of translocation. Schweinburg et al.⁹⁴ injected 5% monoethanolamine oleate intraperitoneally in dogs and then introduced labeled *E. coli* into the stomach. Labeled bacteria appeared in the peritoneal fluid within 24 hours. Morehouse and coworkers¹⁰⁷, using ricinoleic acid in a mouse model, were able to demonstrate increased translocation. Surgical trauma also appears capable of altering the intestinal barrier function to certain macromolecules¹⁰⁸, and thus possibly bacteria. Bombesin, an agent known to stimulate pancreatic and gut mucosal growth has been shown to inhibit bacterial translocation in a rat model¹⁰⁹.

B. Altered Intestinal Microflora

The importance of normal intestinal flora is well recognized, as are disease states produced when it is altered. Pseudomembranous colitis is an example of this. Changes

in intestinal microflora have also been shown to affect the ability of bacteria to translocate. Berg and Garlington⁴ inoculated gnotobiotic mice with the cecal microflora of SPF animals and found bacterial translocation occurred. Furthermore, the ratio of intestinal microflora in the gnotobiotic mice was altered such that coliforms were much more common. In this same experiment, gnotobiotic mice had various individual indigenous strains of microbes introduced into the gut, a process termed monoassociation, and again translocation occurred. The importance of normal flora was further emphasized in another study by Berg¹¹⁰. Germfree mice were monoassociated with *E. coli*, and translocation occurred. Whole cecal contents of SPF mice were then introduced intragastrically into the monoassociated animals and translocation decreased markedly. Examination of cecal contents showed marked reductions in *E. coli*. The importance of viable bacteria in preventing translocation was also examined, and it was found that heat-treated, formalin-treated, or filtered cecal contents did not have the same effect¹¹⁰. Wells et al.¹⁰³ have shown anaerobic flora may be particularly important in this respect.

Whether some bacterial species are more likely to translocate has been examined⁹⁹. *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* were introduced intragastrically into germfree mice and translocation of the different bacteria occurred according to their relative proportion in the cecal contents. In contrast, it is quite clear that while anaerobes are the most common microbes in the intestinal flora, they are rarely found to translocate⁴. Whether this is related to technical difficulties, or attributable to some characteristic of the microbes themselves, is uncertain.

One final study¹¹¹, with interesting clinical applications, has to do with the effect of oral antibiotics on the indigenous gut flora. SPF mice were given four days of either oral penicillin, metronidazole or clindamycin. In each, the cecal flora were found to be changed and the incidence of translocation significantly increased.

C. Decreased Immune Mechanisms

The other major factor in maintaining the gut's ability to resist infection is the immune system. This consists of the general immune system and the gut-associated lymphoid tissue (GALT). The GALT, in turn, is composed of the immunologic cells in the lamina propria, such as B-cells and plasma cells, and the Peyer's patches and lymphoid nodules of the small and large intestine¹⁰².

Several lines of investigation have been used to study the role of the immune system in bacterial translocation. Immunosuppressive agents, such as prednisone and cyclophosphamide¹¹², have been found to promote translocation. However, many of these compounds are also toxic to intestinal epithelium, and thus, these data are merely suggestive of a possible role.

Other investigations involved the use of athymic mice to look at the role of T-cell mediated immunity. Congenitally athymic mice (*nu/nu*) were found to have significantly higher rates of translocation than heterozygotes (*nu/+*)¹¹³. When athymic (*nu/nu*) mice were then grafted with thymus from heterozygotes (*nu/+*), the rate of translocation returned to that of the heterozygotes. Similar results were obtained when neonatal SPF mice were thymectomized¹¹⁴.

In contrast, Maddaus and his colleagues¹⁰² administered cyclosporine, anti-L3T4 antibody, or antithymocyte globulin (ATG) to mice, to study the effect of cell-mediated immunity on translocation. No translocation was observed with up to 14 days treatment. In this same study, T cell-deficient BALB/c nude mice were examined for evidence of bacterial translocation and none was found. This study seems to suggest that T cell immunity is not of primary importance.

It has been conjectured that humoral immunity is important in preventing bacterial translocation. Secretory immunoglobulin A (S-IgA) is the main immunoglobulin in gastrointestinal secretions, and important in preventing attachment of intestinal bacteria to the mucosa¹¹⁵. S-IgA is also found to be markedly reduced by total parenteral nutrition

(TPN)¹¹⁵. Alverdy and his coworkers¹¹⁶, showed in a rat model that TPN resulted in significantly increased bacterial translocation from the gut, and that S-IgA levels were markedly decreased. While this evidence is indirect, it does imply a role for humoral-mediated immunity in preventing translocation.

In summary, the role of the immune system has not been clearly defined with respect to bacterial translocation. Though it appears that depressed immune function promotes bacterial translocation, further studies are required to make definite conclusions.

D. Other Factors Implicated in Bacterial Translocation

Other factors have also been implicated in promoting bacterial translocation. Related to the role of the immune system, is a study in patients with hematological malignancies¹¹⁷. Stool cultures were compared with blood cultures in leukemic patients, undergoing chemotherapy, who had documented gram-negative bacteremia. In over 80% of cases, the dominant strain of *Enterobacteriaceae* or *Pseudomonas aeruginosa* in the stool was identical to that found in the blood cultures. Furthermore, the degree of granulocytopenia was strongly associated with this finding. This evidence cannot be interpreted as proof that translocation occurs in humans, but is suggestive.

The effect of endotoxin on translocation in a mouse model has been studied¹¹⁸. Endotoxin, given either intraperitoneally or intramuscularly, was found to significantly increase the incidence of translocation. The exact mechanism is uncertain, and it should be noted that in this study the cecal flora was significantly altered.

The role of nutrition in bacterial translocation has also received attention. As mentioned above, Alverdy¹¹⁶ showed TPN to increase translocation, though the ratio of normal cecal flora was altered. In the same experiment, TPN, given by mouth, increased incidence of translocation, but again the cecal microflora was found to be altered. Deitch et al¹¹⁹ examined the effect of protein malnutrition in a mouse model. With up to 21 days of protein-free diet, no differences in translocation could be demonstrated. However, it was

shown that the combination of protein malnutrition and endotoxin resulted in a significantly higher incidence of translocation, with higher bacterial counts in mesenteric lymph nodes, and a higher mortality rate versus those animals given endotoxin alone.

Several studies have examined the influence of burns on bacterial translocation. Maejima et al¹²⁰ studied the effects of 15 and 30% burns in a murine model. In SPF mice, no translocation was demonstrated. If SPF mice were monoassociated with *E.coli*, 30% burns resulted in increased translocation. Bacterial translocation has also been demonstrated in a sheep model subjected to thermal and smoke inhalation injuries¹²¹.

E. Bacterial Translocation and Hemorrhagic Shock

With increased interest into the area of bacterial translocation, it was not surprising that the role of the gut in sepsis and hemorrhagic shock be re-examined. Koziol et al.¹²², in a hemorrhaged rat model, were able to demonstrate positive blood cultures within 2 hours of shock. Blood cultures positive during the shock period were monomicrobial, with the commonest organism being *Pseudomonas*. Positive cultures in the post-shock period were polymicrobial. While this model demonstrated bacteremias involving common gut organisms, the source of bacteria was not identified. A subsequent paper¹²³, from the same group, introduced radiolabeled *E. coli* into the gastrointestinal tract of rodents. Radiolabeled bacteria were identified in 7 of 14 shocked rats, versus none of the controls. This study supports the theory of the gut as source of sepsis in hemorrhagic shock.

The same investigators have attempted to relate this work to humans. As discussed above, they were able to demonstrate an increase in the incidence of sepsis of unknown origin in patients in hemorrhagic shock⁹⁰. No one, however, has been able to show conclusively in humans that these organisms arise from the gut. Indeed, this may never be possible.

Little research has been done into the mechanisms by which hemorrhage may promote translocation. As mentioned above, the immune system is abnormal during

shock, perhaps promoting translocation. In addition, changes in splanchnic blood flow in shock may affect the mucosal barrier to bacteria. While changes to intestinal flora are unlikely to occur early on, it is quite conceivable that this may play a role in the long-term critically ill patient.

The only paper to examine a possible mechanism for bacterial translocation in hemorrhagic shock comes from Deitch and his associates¹²⁴. In a rat model, they were able to demonstrate that allopurinol, a xanthine oxidase inhibitor, caused a reduction in the incidence of bacterial translocation versus controls. This was also shown in rats on a tungsten-supplemented molybdenum-free diet, which inactivates xanthine oxidase. Since xanthine oxidase-generated oxygen free radicals have been implicated as one of the mediators in intestinal ischemia⁶³, this would suggest a possible mechanism.

F. The Role of the Lymphatic System in Bacterial Translocation

From the time of Asellius, and his demonstration of lymphatics in a fed dog¹²⁵, the importance of the intestinal lymphatics in gut absorption has been recognized. While most studies of translocation have examined the mesenteric lymph nodes, the lymph itself has not been examined. Thus the question remains, do bacteria translocate into the vascular system or into the lymphatic system?

Investigators have been able to demonstrate the ability of intestinal lymphatics to absorb proteins and other macromolecules in animal models^{126,127,128}. To date, only two studies have looked at the bacterial content of lymph in hemorrhagic shock. The first⁹², one of the original studies by Jacob et al., examined thoracic duct drainage in a dog model, and were unable to culture any bacteria from the lymph. The second¹²⁹ compared thoracic duct lymph to portal blood in a dog model. Though bacteria were demonstrated in the portal blood, no bacteria were identified in the thoracic lymph. Both of these suffered in that thoracic duct drainage may not represent prenodal lymph¹³⁰. It would seem then, that the role of the lymphatic system in bacterial translocation has been relatively ignored.

Summary

The pathophysiology of hemorrhagic shock has been selectively reviewed. Specifically, the role of the gut as a source of sepsis in hemorrhagic shock has been examined. A historical perspective of the field has been given, followed by a review of the current theory which attempts to explain the association between hemorrhage and sepsis, bacterial translocation. While this theory is an interesting one, and holds promise for the future, major questions remain unanswered.

Most studies of translocation and hemorrhage have been in rodent models, and indeed translocation, under any circumstance, has only been shown in one large animal study. This in itself, is of concern. As Fine and coworkers realized, all models have limitations. In the case of the dog, the problem of a continually septic splanchnic circulation was a major obstacle, as was the unusual contractile nature of the hepatic vein. Similarly, rodents are a less than perfect model for studying hemorrhagic shock. Rats and mice have also been shown to have sphincter-like mechanisms around the effluent hepatic veins which contract with hypotension, and have splanchnic beds which are very sensitive to vasoactive compounds¹³¹. No model is perfect, and extrapolation of data from rats to humans is not always appropriate. This is especially pertinent in the case of bacterial translocation and shock, where documentation in man may be impossible.

Given these limitations, this study set out to demonstrate translocation in a large animal model which is generally recognized as being more clinically relevant; the pig. These animals meet several basic requirements of any model animal, in that they are easily available and can be handled without difficulty. More importantly, the cardiovascular system¹³² and gastrointestinal tract¹³³ are felt to closely mimic that of man, from a physiologic standpoint. Indeed, swine are now commonly used in studies of hemorrhagic shock, and have been shown to closely correspond to the situation in man¹³⁴.

A secondary objective of this study was to learn something of the mechanisms whereby hemorrhagic shock promotes translocation. At present, it is not known whether translocation is an injury which occurs with ischemia or reperfusion. Because of a limitation of time and funding, only the ischemic event itself was examined: work in the reperfusion period must await further study. Finally, it is unknown if bacteria gain access via the portal circulation, or through the extensive lymphatic network associated with the gut. Given the ability of this laboratory to cannulate mesenteric lymphatic vessels, this was an excellent opportunity to investigate if translocation occurs directly into the mesenteric lymphatics.

MATERIALS AND METHODS

I. Animal Preparation

Twenty-four male swine between 26 - 43 Kg were used in this study. A cross between Landrace and White York, all were obtained from one supplier (M. Borys, Leduc, Alberta), and were not on antibiotics. Animals were housed at the Biomedical Animal Facility for several days prior to the experiment, and brought to the main facility (Surgical Medical Research Institute) the day before surgery. All were deprived of food and water for twelve hrs prior to operation. The morning of surgery, each was prospectively and randomly assigned to either the control or experimental group according to a list prepared by an independent, blinded source (Dr. M. Grace).

An anaesthetic routine was developed to provide adequate anaesthesia and analgesia, and based on a regimen developed by another investigator¹³⁵. Ketamine Hydrochloride (Ketalean, 25 mg/Kg, MTC Pharmaceuticals, Hamilton, Ontario) and Atropine Sulfate (Atropine, 0.05 mg/Kg Abbott Laboratories Limited, Montreal, Quebec) were given as an intramuscular injection in the hind leg. Animals were induced with Halothane (Halothane, 4%, Halocarbon Laboratories Incorporated, Hackensack, New Jersey) by face mask, and an intravenous catheter (Flash-Cath, # 20 Gauge, Travenol Laboratories Inc., Deerfield, Illinois) inserted in an ear vein and secured. During the operation, normal saline (0.9% Saline, Travenol Canada Inc., Mississauga, Ontario) was administered at a rate of 10 ml/Kg/hr by an automated volumetric infusion pump (Imed 927, Imed Ltd., England).

The animals were placed, in the supine position, on the operating table, and intubated with an endotracheal tube (Portex, 6.0 - 7.0). Auscultation was used to assure correct tube placement. The dose of Halothane was then decreased to 1% for the duration of surgery, and the animal placed on a pressure ventilator (Fraser Harlake Anesthesia Ventilator, Fraser Sweatman Incorporated, Orchard Park, New York). Tidal volume was maintained at 10-15 ml/Kg¹³⁶, respiratory rate at 12-15 b/minute¹³⁶, and inspiratory to

expiratory ratio at 1:2. Assisted ventilation was maintained for the duration of the experiment, with respiratory rate adjusted according to results of arterial blood gas determinations. The fraction of inspired oxygen (FiO_2) was adjusted throughout the experiment to maintain the partial pressure of oxygen in arterial blood (P_aO_2) between 150-200 mm Hg. An effort was made to keep the partial pressure of carbon dioxide of arterial blood (P_aCO_2) between 40-45 mm Hg, the generally agreed upon value for swine¹³⁷, by adjusting the respiratory rate and tidal volume.

Electrocardiograph leads were placed subcutaneously in the right and left foreleg, and the left hindlegs. The leads were fed to a preamplifier (Model 78203C, Hewlett Packard), then fed to a stripchart recorder (R611, Beckman Instruments Incorporated, Schiller Park, Illinois) and heart rate recorded. The abdomen and right groin were shaved and washed with a 7.5% povidine-iodine surgical scrub (Betadine Surgical Scrub, Purdue Frederick, Toronto, Ontario). The area was painted with a 10% solution of povidine-iodine (Betadine Solution, Purdue Frederick, Toronto, Ontario) and draped in a sterile manner.

II. Surgical Procedures

A. Aseptic Technique

The nature of this study demanded aseptic technique at all times, and unless otherwise mentioned, can be assumed. All equipment in contact with the animal was sterilized prior to use by one of three methods. New equipment in sealed packages was already sterilized, usually by ionizing radiation. All other equipment was carefully washed with soap and water, and packaged. Heat-resistant equipment was steam sterilized at 121 °C for one hr. Plastics, and other heat-sensitive materials were gas sterilized with ethylene oxide for one hour and forty-five minutes, and then aerated for twelve hrs. This equipment

was carefully flushed prior to its use with copious amounts of sterile saline to remove any remaining gas.

B. Femoral Artery and Vein Catheterization

An oblique incision was made in the right groin, and the femoral artery and vein exposed. The artery was dissected free, proximal and distal control obtained, and the vessel ligated distally using cotton ties (2-0 Cotton, Davis and Geck, Montreal, Quebec). The arterial catheter (Arrow Percutaneous Sheath Introducer, 8.5F, Arrow International Incorporated, Reading, Pennsylvania)) was inserted through a small transverse incision. It was connected to a pressure transducer (Model 1290A, Hewlett Packard), a preamplifier (Model 78205B Hewlett Packard) and then fed to another channel of the stripchart recorder. A dilute solution of Heparin Sodium (Allen and Hanburys, 1000 unit/ml, Toronto, Ontario), made up to a final concentration of 1 unit/ml, was infused at a rate of 3 ml/hr to maintain catheter patency.

The femoral vein was isolated in similar fashion, and a catheter (Cordis Catheter Sheath Introducer System - 8F, Cordis Corporation, Miami, Florida) inserted. Again, a dilute Heparin Sodium solution was used to prevent catheter clotting. The incision was closed with a running suture of braided polyglycolic acid (0 Dexon Plus, Davis and Geck, Montreal, Quebec).

C. Abdominal Operation

A midline incision was made, the peritoneal cavity entered, and the urinary bladder identified and delivered to the wound. Two purse string sutures of braided polyglycolic acid (2-0 Dexon Plus, Davis and Geck, Montreal, Quebec) were inserted in the apex of the bladder, and a small incision made with electrocautery. A foley catheter (16 Fr, Bard Canada Incorporated, Mississauga, Ontario) was inserted, its balloon inflated with 10 ml of saline, and the purse string sutures tied to secure the catheter in place. A small incision was

made at the inferior aspect of the abdominal wound, the foley catheter brought to the surface and connected to a graduated urine collection bag.

The small bowel was then delivered to the wound. The craniomesenteric artery was identified, and a small portion cleared completely of surrounding tissue. An electromagnetic flow probe (Model CF412H, Carolina Medical Electronics Incorporated, King, North Carolina) of appropriate size was zeroed, and then positioned around the artery. The probe was connected to a flowmeter (CME Cliniflow 601 - D, Carolina Medical Electronics Incorporated, King, North Carolina), which was in turn fed to a third channel of the stripchart recorder. The flowmeter and probes were calibrated prior to the study, according to the technique recommended by the manufacturer. This involved harvesting a femoral vessel, positioning the appropriate probe around it, and then running heparinized whole blood through the vessel at a known rate. Flow probe measurements corresponded to within 5% of the actual flow rates.

The portal vein was identified, and a branch between 3 - 5 mm selected and isolated. Proximal and distal control was obtained and the distal end ligated. A transverse incision was made, and a triple-lumen catheter (Multimed Catheter - 703K200H -7F, Baxter Healthcare Corporation, Irvine, California) inserted approximately 10 cm into the vein, such that all three distal openings were lying well within the main portal vein. This catheter was also kept patent with a dilute heparin sodium solution (1 unit/ml).

The final stage of the abdominal operation was the insertion of a catheter into an efferent mesenteric lymphatic vessel. These vessels drain the mesenteric lymph nodes towards the cisterna chyli. A suitable 1 - 2 mm diameter vessel was identified and ligated proximally with a silk ligature (4-0 Silk, Ethicon Sutures Limited, Peterborough, Ontario). The vessel was allowed to dilate, a transverse incision made, and a polyethylene catheter (Intramedic PE-160, Outer Diameter - 1.57 mm, Clay Adams, Parsippany N.J.) inserted and secured. The distal end was brought out through the abdominal incision, and placed into a graduated test-tube through a hole in the test-tube cover. Thus, lymph from the

mesenteric lymphatic vessel was collected. All test-tubes had been previously calibrated with a graduated cylinder.

At this point, the halothane anesthetic was discontinued, and the animal placed on an intravenous infusion of Sodium Pentobarbital (Somnotol, MTC Pharmaceuticals, Cambridge Ontario) according to the following protocol (Dr. R. Mustard, personal communication):

1. Sodium Pentobarbital - 40 mg/Kg for 30 minutes.
2. Sodium Pentobarbital - 20 mg/Kg for 60 minutes.
3. Sodium Pentobarbital - 5 - 10 mg/Kg/hr for the duration of the experiment.

Pentobarbital was infused by an automated volumetric infusion pump to provide constant rates.

The abdomen was closed in two layers, using running sutures of braided polyglycolic acid for the fascia (0 Dexon Plus, Davis and Geck, Montreal, Quebec), and skin (2-0 Dexon Plus, Davis and Geck, Montreal, Quebec). Once closed, the normal saline infusion was decreased to maintenance levels calculated according to the formula¹³⁵:

$$\text{IV Maintenance Fluid/24 hrs} = 40 \text{ ml/Kg.}$$

This rate was kept constant for the rest of the experiment.

A flow directed thermodilution catheter (Swan-Ganz 93A-131-7F, American Edwards Laboratories, Anasco, Puerto Rico) was inserted through the femoral venous catheter. It was connected to a pressure transducer (Model 1290A, Hewlett Packard), a preamplifier (Hewlett Packard 78205B), and then to a stripchart recorder (Beckman 611). The catheter tip was flow-directed into the pulmonary artery until ascertained to be in the 'wedged' position, according to pressure wave recordings. The catheter was also connected to a cardiac output computer (model 9250A, Edwards Laboratories, Santa Ana, California) to measure core temperature and cardiac output.

III. Postoperative Monitoring

The animal was allowed to stabilize for one hour after the abdomen was closed. In all instances, the final sodium pentobarbital rate of 5-10 mg/Kg/hr had been infusing for at least one hour. At this time, arbitrarily called T=0 hr, monitoring was begun. Animals were followed for the next hour, during which baseline hemodynamic and biochemical measurements were made, and cultures taken from the portal blood and mesenteric lymph. At T=1 hr, the animals entered either the control or experimental group. The experimental group was bled 40% of the estimated blood volume over a 30 minute period. This value was determined from the formula¹³⁸:

$$\text{Estimated Blood Volume (ml)} = (179 \times W^{-0.27}) \times W$$

Where: W = Weight (Kg).

The control group was observed for the same 30 minute period. All animals were followed for the ensuing six hours, or until death occurred. Monitoring was considered in three general categories, hemodynamic, biochemical and hematological, and bacteriological. The time scale of the overall experiment is shown (Figure 1).

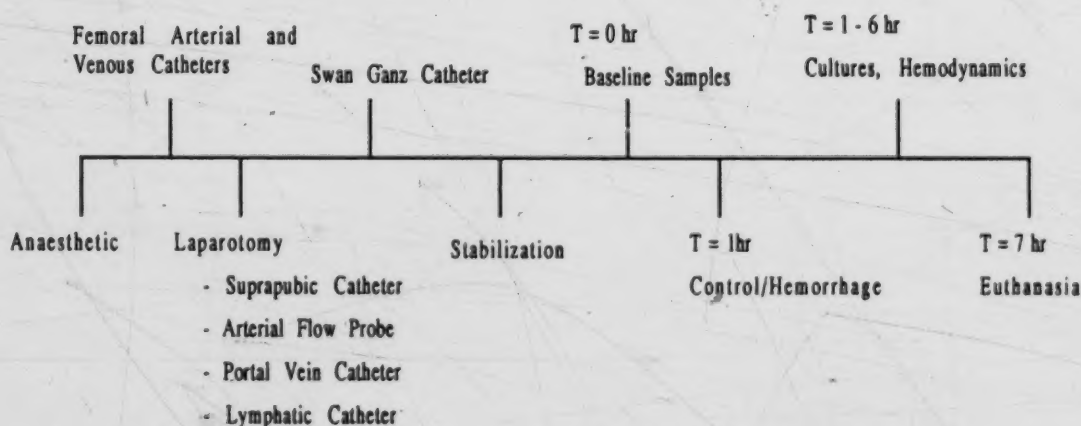


Figure 1 - Experimental Time Schedule - Abbreviated

In general, measurements are given in SI units. However, in instances where SI units have little clinical use, this has been purposely omitted. An example of this would be reporting mean arterial pressure in mm Hg. To report this information in kilopascals, the proper SI unit for pressure, would be in marked contrast to the clinical setting in North America and the world literature¹³⁹.

A. Hemodynamic Measurements

The following measurements were taken at specified intervals (Table 1) and considered under the general heading of hemodynamic measurements:

Table 1 - Hemodynamic Parameters and Time Schedule of Measurements

Parameter	Time (hours)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
H R	X	X	X	X	X	X	X
MAP	X	X	X	X	X	X	X
RAP	X	X	X	X	X	X	X
PADP	X	X	X	X	X	X	X
PWP	X	X	X	X	X	X	X
CI		X		X		X	X
LF	X	X		X	X	X	X
CAF	X	X	X	X	X	X	X
U/O	X	X		X	X	X	X
IV's In	X	X		X	X	X	X
Temp.	X	X		X	X	X	X
R R	X	X		X	X	X	X
Weight	X						

1. Heart rate (H R) was measured as described above, and reported as beats per minute (bpm).

2. Mean Arterial Pressure (MAP) was calculated using the formula:

MAP = diastolic pressure + $1/3(\text{systolic} - \text{diastolic pressure})$, and reported in mm Hg.

3. Right Atrial Pressure (RAP) was measured from the proximal port of the Swan-Ganz catheter and reported in mm Hg.
4. Mean Pulmonary Artery Diastolic Pressure (PADP) was measured from the distal port of the Swan Ganz catheter with the balloon deflated and reported in mm Hg.
5. Pulmonary Wedge Pressure (PWP) was taken as the mean pressure measurement from the distal port of the Swan Ganz catheter with the balloon inflated and reported in mm Hg.
6. Cardiac Output (CO) was calculated using the Swan-Ganz catheter, the cardiac output computer, and the thermodilution technique. Aliquots of 5% Dextrose solution kept between 0 - 5 °C (Travenol Canada Inc., Mississauga, Ontario) were used. Four measurements made for each sample period, and the average taken. Cardiac output was divided by the weight of the animal, in Kg, to give Cardiac Index (C I), in ml/min/Kg, conforming with other studies in this area¹⁴⁰.
7. Lymph Flow (LF) was measured by collecting the lymph in calibrated, graduated test-tubes, and dividing by the length of the collection period. Flow was expressed in ml/hour.
8. Craniomesenteric Arterial Flow (CAF) was measured using the electromagnetic flow probe already described. Data was reported in ml/min/Kg, by dividing by the measured flow by weight, in keeping with the literature¹⁴⁰.
9. Urine Output (U/O) - Urine from the indwelling catheter was collected and measured in a calibrated urine collection bag. The values were reported in ml/hour.

10. Respiratory Rate (RR), in breaths per minute (b/min), was measured with the aid of a chain and an expandable tubing positioned around the animals thorax. This was then connected to a pressure transducer (Model P23D6, Gould Stratham), and fed into a separate channel on the stripchart recorder. Respiratory movements resulted in changes of volume within the tubing, and this was detected by the pressure transducer.
11. Intravenous fluids administered (IV's In) were recorded, and reported in ml/hr.
12. Temperature (Temp), in degrees Celsius ($^{\circ}\text{C}$), was recorded from the Swan-Ganz catheter, and read from the cardiac output computer. A heating blanket was positioned under the animal prior to surgery, and the temperature kept between 38.5 and 39.5 $^{\circ}\text{C}$. The normal resting temperature of a pig is 39 $^{\circ}\text{C}$ ¹⁴¹.
13. Weights were measured the morning of surgery, using the same scale each time, and reported in Kg.

B. Biochemical and Hematological Measurements

Blood and lymph samples were taken at regular intervals (Table 2) and the following measurements made:

Table 2 - Biochemical Parameters and Time Schedule of Measurements

Parameter	Time (hours)					
	T=0	T=1	T=2	T=3	T=5	T=7
PaO ₂	X	X	X	X	X	X
PaCO ₂	X	X	X	X	X	X
[H ⁺]	X	X	X	X	X	X
HCO ₃	X	X	X	X	X	X
PvO ₂	X	X	X	X	X	X
Hg	X	X	X	X	X	X
Hct	X	X	X	X	X	X
TP	X	X	X	X	X	X
Albumin	X	X	X	X	X	X
SaO ₂	X	X	X	X	X	X
SvO ₂	X	X	X	X	X	X
CaO ₂	X	X	X	X	X	X
CvO ₂	X	X	X	X	X	X
Ca-vO ₂	X	X	X	X	X	X

1. Partial pressure of Oxygen of arterial blood (P_aO₂) was measured from samples obtained from the femoral artery, and reported in mm Hg. This blood was drawn into a heparinized syringe, and kept on ice until run through an electrode type automated pH and blood gas analyzer (IL 813, Instrumentation Laboratory Incorporated, Lexington, Massachusetts). Samples were usually run within 15 minutes of drawing.
2. Partial pressure of Carbon Dioxide of arterial blood (P_aCO₂) was measured from the same sample, using the same apparatus, and reported in mm Hg.
3. Hydrogen ion concentration of arterial blood ([H⁺]) was calculated from the same femoral sample, using the same blood gas analyzer. The machine reported results in pH, and this was converted to [H⁺], in nmol/L, using the formula:

[H+]=antilog pH.

4. Bicarbonate (HCO_3), in mmol/L, was calculated from the femoral arterial sample by the blood gas analyzer.
5. Mixed venous samples obtained from the distal port of the pulmonary artery catheter were drawn in heparinized syringes and run through the same blood gas analyzer. This allowed measurement of the mixed-venous oxygen (PvO_2), which was reported in mm Hg.
6. Hemoglobin (*Hg*), in grams per deciliter (g/dl), was measured from the arterial sample only, using an automated photoelectric device (Hemoglobinometer, Coulter Electronics Incorporated, Hialeah, Florida).
7. Hematocrit (*Hct*) was measured from the arterial sample only. The sample was centrifuged (Readacrit Centrifuge, Clay Adams, Parsippany, New Jersey) and the result read directly.
8. Total Protein (*TP*), in g/dl, was measured from both arterial and lymph samples. This was done using an automated spectrophotometer and microcentrifuge (Multistat III, Instrumentation Laboratory Incorporated, Lexington, Massachusetts). The reagent (Liquid-STAT Total Protein Reagent, Beckman Instruments, Carlsbad, California) used is based on the biuret reaction. This method recognizes the specific configuration of human protein, and thus is not as accurate for swine. Electrophoresis would have been a more desirable method of measuring TP, however its considerable expense prevented its use. Other investigators have found that Multistat results are adequate to show trends in total protein¹⁴².
9. Albumin concentration, in g/dl, was obtained from both arterial and lymph samples. Samples were run on the same automated spectrophotometer and microcentrifuge as used for TP, however a different reagent was used. This reagent (Beckman Albumin Reagent, Beckman Instruments, Carlsbad,

California) depends upon a bromocresol dye reaction with albumin. Again, these values are not identical with those obtained by electrophoresis, for similar reasons. However, they too are able to demonstrate trends with reasonable accuracy¹⁴².

10. Arterial oxygen saturation (S_aO_2), in %, was calculated using the Hill Equation¹⁴³:

$$S_aO_2 = \frac{(P_aO_2/P_{50})^n}{1 + (P_aO_2/P_{50})^n} \times 100$$

Where: P_{50} = Partial Pressure of Oxygen at Which Hemoglobin is 50% Saturated = 35.7 mm Hg at 37°C, pH 7.4 in the swine¹⁴⁴
 n = Hill Coefficient = 2.94 at P_{50} , 37°C, pH 7.4¹⁴⁴

11. Venous oxygen saturation (S_vO_2), in %, was calculated in a similar manner, substituting P_vO_2 for P_aO_2 .
12. Oxygen content of arterial blood (C_aO_2), in ml O_2 /dl, was calculated using the formula:

$$C_aO_2 = (1.34 \times S_aO_2 \times Hg) + (0.0031 \times P_aO_2)$$

Where: 1.34 = Amount of Oxygen Bound to Hemoglobin When Fully Saturated (in ml O_2 /g Hg)
 0.0031 = Amount of Oxygen Dissolved in Blood (in ml O_2 /dl/mm Hg)

13. Oxygen content of mixed venous blood (C_vO_2), in ml O_2 /dl, was calculated using the same formula, substituting P_vO_2 for P_aO_2 , and S_vO_2 for S_aO_2 .
14. The arterial-mixed venous oxygen content difference ($C_{a-v}O_2$), in ml O_2 /dl, was calculated using the formula:

$$C_{a-v}O_2 = C_aO_2 - C_vO_2$$

C. Bacteriological Monitoring

Samples of portal blood and mesenteric lymph were taken at T=1 (prebleed), T=2 hours (one hour postbleed), T=5 hours (four hours postbleed) and T=7 hours (six hours postbleed) were taken for culture. Previous studies have shown that over 80% of bacterial translocation in other shock models occurs within 5 hours of hemorrhage¹²². Portal blood was obtained from the portal vein catheter, having first removed any heparin in the catheter. Aliquots of 1.5 ml were injected into aerobic (Bactec NR6A Culture Vial, Becton Dickinson Diagnostic Instruments System, Towson, Maryland) and anaerobic (Bactec NR7A Culture Vial, Becton Dickinson Diagnostic Instruments System, Towson, Maryland) culture vials. In a similar fashion, 0.5 ml aliquots of mesenteric lymph were sent for aerobic and anaerobic culture. In order to prevent isolated portal blood bacteremia from being falsely interpreted as systemic bacteremia, a systemic blood sample was taken at T=7 hours for aerobic and anaerobic culture. The sample was obtained from the superior vena cava via a sterile cutdown in the neck of the animal. Again, 1.5 ml aliquots were placed into anaerobic and aerobic culture vials. Systemic samples were not taken for the rest of the intervals used for portal blood and lymph. This was based partly on the reasoning that systemic bacteremia, if it was going to occur, would be most likely to occur longer periods after shock, and partly on the very great expense of performing these additional cultures.

All culture vials were then taken to the Microbiology Laboratory at the University of Alberta Hospital, and placed through an automated blood culture analyzer (Bactec 660, Becton Dickinson Diagnostic Instruments System, Towson, Maryland). This self contained unit incubated all samples at 37 °C. Each vial was sampled to determine the amount of carbon dioxide produced, and if significant, the vial opened and plated on the following media:

1. Blood Agar in Carbon Dioxide
2. Brain heart Infusion
3. Chocolate Agar

In addition, a gram stain was performed. All vials were sampled twice a day for the first forty-eight hours, and then once a day for the next five days. Lack of significant carbon dioxide production by this time, was interpreted as no growth. All positive cultures were read by one individual (Dr. P. Kibsey), who was blinded as to which group the animal was assigned.

After all other samples and measurements were made, the abdomen was again scrubbed and painted with povidine. The sutures were cut and the peritoneal cavity entered. Mesenteric lymph nodes near the lymph catheter were identified, and the overlying peritoneum excised. Then, using a separate pair of forceps and scissors, lymph nodes were excised and placed in a sterile container.

The node was processed by the Microbiology Laboratory of the University of Alberta Hospital. The node was ground in a sterile fashion and plated onto two media:

1. Blood Agar in Carbon Dioxide
2. McKonkey Agar

A gram stain was done. Plates were observed for three days. The same process for identification was used for the lymph nodes as for the other cultures.

D. Bowel Biopsy

In order to assess the effects of hemorrhage on the bowel itself, samples of the terminal ileum were taken at the time of the lymph node recovery. These were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. The slides were all examined by one pathologist (Dr. L. Jewell), who was blinded as to whether the animal was bled or not.

E. Euthanasia

Unless already dead, the animals were sacrificed after the bowel specimen had been obtained with an intravenous infusion of saturated potassium chloride. Death usually occurred within 30 seconds of infusion.

F. Statistical Method

All laboratory data was coded and entered into the computer. Information from the data base was edited and analysis was done by the SPSS on the University of Alberta mainframe computer. Descriptive statistics and frequencies were determined for each treatment group. Comparison between groups at baseline were performed both with t-tests and Fisher's exact test. Primary comparisons were completed at T=0 hr, T=1hr, T=1.5 hr, T=2 hr, T=3 hr, T=5 hr, and T=7 hr. Only T=1 hr, T=2 hr, and T=7 hr were reported in the analysis. Comparisons were made between the groups by a t-test and Fisher's exact test and within groups by a paired t-test. The level of significance was taken as $p < 0.05$.

RESULTS

I. Inclusion vs. Exclusion

Twenty-four animals were entered in this study, with four excluded. In each instance, these animals were removed because they failed to complete the experimental protocol, for reasons other than death due to hemorrhage. In the first, multiple attempts at cannulating the lymphatic vessels were unsuccessful, and the animal euthanized. In the second, the pig possessed a remarkably long neck, and intubation was extremely difficult. During intubation, a tracheal injury occurred, which was undetected until well into the operation. Unable to ventilate the animal, a tracheostomy was performed, however the animal became hypotensive and died. The third and fourth animals died due to technical error. In each instance the oxygen tank ran out requiring changing. Though these were changed quickly, both animals, having already been bled, were extremely sensitive and died of hypotension within one or two minutes. In most instances, minimal data was available for the excluded group. Weight and initial hemoglobin were recorded for these four animals (Table 3), and for those entered into the study (Table 4). Excluded animals had an average Wt of 32.0 ± 3.6 Kg, compared to 31.7 ± 3.5 Kg for those included. Initial hemoglobins were 12.3 ± 1.9 g/dl and 11.1 ± 1.5 g/dl respectively. These differences were not significant.

Table 3 - Weight and Initial Hemoglobin of Excluded Animals

Pig #	Wt(Kg)	Hg (g/dl)
14	30.2	10.9
16	36.0	14.5
21	33.8	NA
25	28.0	11.5
Mean	32.0	12.3
Std. Dev.	3.6	1.9

Table 4 - Weight and Initial Hemoglobin of Included Animals

	Wt (Kg)	Hg (g/dl) at T=0
Average	31.7	11.1
Std. Dev.	3.5	1.5

II. Mortality

A total of twenty animals were randomly assigned to control and experimental groups of equal size (n=10). Mortalities were considered to be animals who failed to complete the entire experiment (Table 5). All animals in the control group survived the six hr observation period and were euthanized. Three of ten experimental animals died, between T=5 and T=7 hrs. In each instance, the animal became profoundly hypotensive. The difference in mortality between the two groups was not significant.

Table 5 - Outcome of Control and Experimental Animals

Control Pigs	Survival	Experimental Pigs	Survival
15	y	13	n
17	y	19	y
18	y	22	y
20	y	24	y
23	y	27	n
26	y	30	y
28	y	31	y
29	y	32	y
33	y	34	n
35	y	36	y
Total	10	Total	7

III. Hemodynamics

In dealing with this general category, areas which can be considered as relatively independent of the experiment will be discussed first. This includes Wt, intravenous fluids

infused, and urine output prior to T=0 hr. All other hemodynamic results will then be considered.

A. Weight

The Wts of control and experimental animals are given (Table 6). Control animals had a Wt of 31.1 ± 1.0 Kg, and experimental animals had a Wt of 32.3 ± 4.8 Kg. These differences were not statistically significant.

Table 6 - Weight of Control and Experimental Animals

Control Animals		Experimental Animals	
Pig #	Wt (Kg)	Pig #	Wt(Kg)
15	32.0	13	33.2
17	29.0	19	27.7
18	30.7	22	33.2
20	31.4	24	28.0
23	30.8	27	31.8
26	30.4	30	33.4
28	32.2	31	34.1
29	31.2	32	31.6
33	31.7	34	43.7
35	32.0	36	26.4
Average	31.1	Average	32.3
Std. Dev.	1.0	Std. Dev.	4.8

B. Intravenous Fluids

The volume of intravenous fluids administered to control (Table 7) and experimental (Table 8) animals are given. The volume given animals prior to the observation, listed as To T=0, did not differ between the two groups. Controls received 1180 ± 199 ml compared with 1094 ± 147 ml for experimental animals for this time period. The two groups did not differ for the volume of fluids given over the rest of the experiment.

Table 7 - Volume of Intravenous Fluids (ml) Administered to Control Animals By Time

Pig #	Time (hrs)					
	To T=0	T=0	T=1	T=2	T=3	T=5
15	1008	60	55	59	66	63
17	1046	58	57	57	58	59
18	1197	57	58	60	56	57
20	1337	67	67	67	66	66
23	1066	78	67	67	68	59
26	1064	66	65	65	67	67
28	1625	63	68	64	64	68
29	1046	72	69	68	65	63
33	1062	69	69	63	68	68
35	1349	68	65	65	65	63
Average	1180	66	64	64	64	63
Std. Dev.	199	6.5	5.3	3.7	4.1	4.0

Table 8 - Volume of Intravenous Fluids (ml) Administered to Experimental Animals By Time

Pig #	Time(hrs)					
	To T=0	T=0	T=1	T=2	T=3	T=5
13	1275	76	120	63	63	88
19	878	56	69	69	55	55
22	967	65	91	63	66	63
24	1054	64	303	124	75	66
27	1033	78	522	60	60	NA
30	1093	67	56	68	65	67
31	1204	67	63	63	66	63
32	1074	63	62	60	60	60
34	1362	94	78	90	95	70
36	999	56	51	50	50	52
Average	1094	69	142	71	66	65
Std. Dev.	147	11.4	153.2	21.2	12.4	10.4

C. Urine Output Prior To Observation

Urine output up to T=0 hr is an indication of the degree of hydration of the animals, and independent of whether or not the animal was bled. For this reason, this data will be considered separately from the rest of the data on urine output.

Table 9 shows urine output prior to T=0 hr for control animals, and Table 10 the same data for experimental animals. In both tables, urine output is divided by body weight to standardize results. Control animals had an output of 8.1 ± 5.2 ml /Kg body Wt. prior

to T=0 hr, whereas experimental animals put out 8.8 ± 3.4 ml/Kg body Wt. over the same period. These values did not differ significantly.

Table 9 - Urine Output Up to T=0 hr for Control Animals

Pig #	Urine Output To T=0 (ml)	Urine Output/Wt To T=0 (ml/Kg)
15	80	2.5
17	115	4.0
18	240	7.8
20	150	4.8
23	570	18.5
26	252	8.3
28	276	8.6
29	500	16.0
33	185	5.8
35	150	4.7
Average	252	8.1
Std. Dev.	162	5.2

Table 10 - Urine Output Prior To T=0 hr For Experimental Animals

Pig #	Urine Output To T=0 (ml)	Urine Output/Wt To T=0 (ml/Kg)
13	300	9.0
19	230	8.3
22	275	8.3
24	290	10.4
27	135	4.2
30	135	4.0
31	480	14.1
32	281	8.9
34	302	6.9
36	365	13.8
Average	279	8.8
Std. Dev.	102	3.3

D. Heart Rate

Heart rates during the experiment are given for control (Table 11) and experimental (Table 12) groups, and depicted graphically (Figure 2). For control animals, the HRs at T=0 and T=1 hrs do not differ significantly within the group, as is the case for experimental animals. Comparing the two groups immediately prior to hemorrhage, at T=1 hr, controls have a HR of 143 ± 28 bpm, and experimentals a rate of 145 ± 34 bpm. These differences are not significant. Over the course of the experiment, controls show a slight increase to 145 ± 34 and 157 ± 29 bpm at T=2 and 7 hrs respectively. In contrast, the experimental group showed a sudden, profound and persistent tachycardia. At T=2 and T=7 hrs, HRs were 241 ± 28 and 211 ± 32 bpm, and these were significantly greater than controls ($p < 0.0001$ and $p < 0.05$).

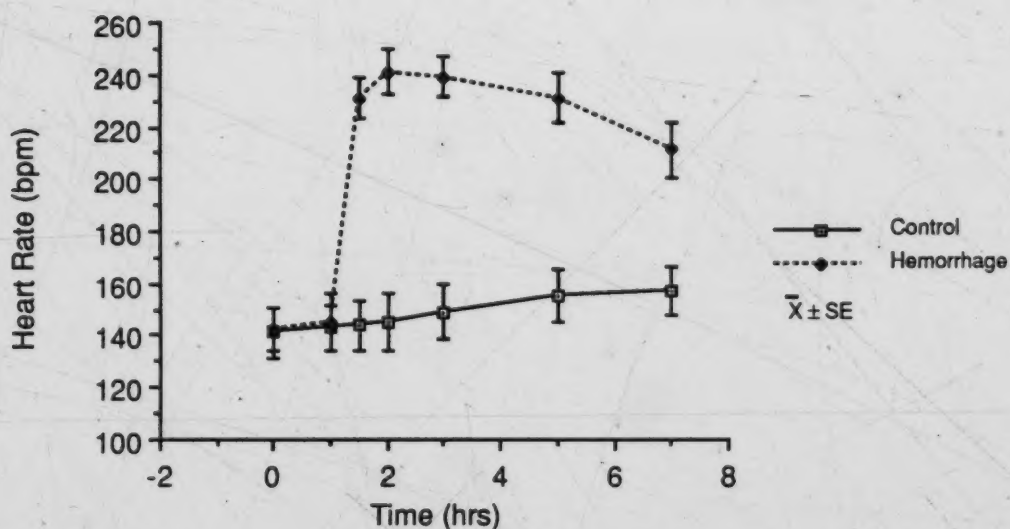


Figure 2 - Heart Rate for Control and Experimental Animals

Table 11 - Heart Rate (bpm) for Control Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
15	108	115	112	96	108	115	115
17	163	170	184	185	189	180	170
18	107	105	108	106	105	104	107
20	126	132	135	136	134	145	165
23	106	112	108	114	114	134	144
26	176	176	180	172	180	180	180
28	152	160	156	168	160	168	156
33	180	180	180	196	198	204	204
35	120	132	132	132	144	144	160
Average	141	143	144	145	149	155	157
Std. Dev.	30	28	30	34	34	32	29
SEM	9.6	8.8	9.5	10.9	10.7	10.1	9.2

Table 12 - Heart Rate (bpm) for Experimental Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
13	123	124	220	225	225	194	145
19	127	124	195	206	213	215	220
22	113	112	237	238	242	246	243
24	152	160	235	268	252	248	212
27	200	224	276	300	288	288	NA
30	140	136	196	220	240	228	224
31	120	124	216	220	210	192	186
32	150	144	240	224	216	204	198
34	162	176	252	244	264	228	228
36	132	128	244	260	240	266	246
Average	142	145	231	241	239	231	211
Std. Dev.	26	34	25	28	25	31	32
SEM	8.1	10.7	7.9	9.0	7.8	9.9	10.5

E. Mean Arterial Pressure

Mean systemic arterial pressure for controls (Table 13) and experimental (Table 14) animals are given, and means and standard errors shown (Figure 3). Results for the baseline hour do not differ significantly within either group. Immediately prior to hemorrhage, the control group had an MAP of 94 ± 8.4 mm Hg, versus 102 ± 11.9 mm Hg for the experimental group and these differences were not significant. The control group showed a drop in pressure over the next hour, to 90 ± 6.5 mm Hg, which differed significantly from the baseline value ($p < 0.05$). However, this was not the case for the rest of the experiment, and MAPs did not differ significantly from the baseline values, being 90 ± 6.5 mm Hg at T=2 hr and 89 ± 6.4 mm Hg at T=7 hr. In contrast, the experimental group showed profound hypotension after hemorrhage. Mean arterial pressures at T=2 and 7 hrs were 61 ± 13.6 and 62 ± 26.5 mm Hg. These were significantly lower than the control group ($p < 0.0001$ and $p < 0.005$ respectively).

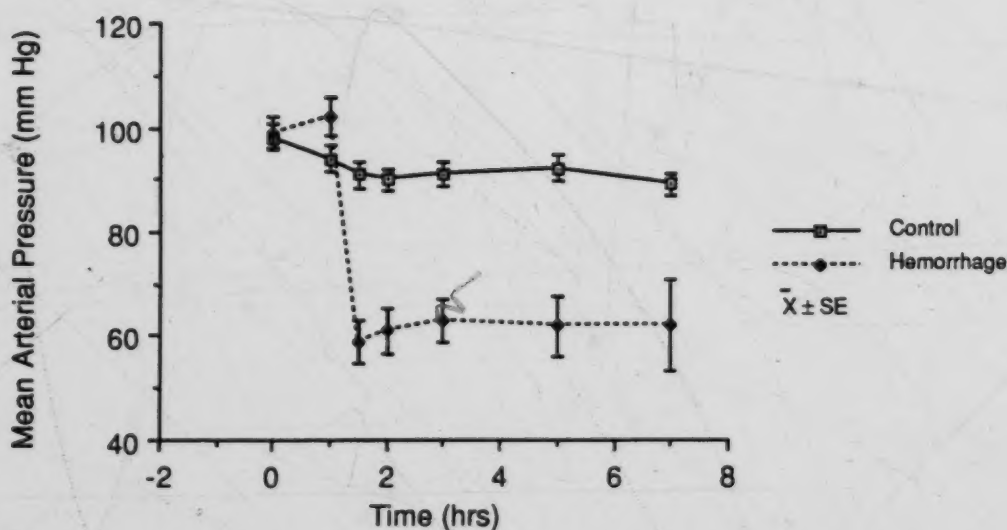


Figure 3 - Mean Arterial Pressure for Control and Experimental Animals

Table 13 - Mean Arterial Pressure (mm Hg) for Control Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
15	93	99	92	92	97	95	99
17	108	96	85	85	95	106	90
18	97	95	90	87	89	92	91
20	99	103	102	97	92	86	85
23	104	108	104	99	96	90	88
26	95	84	92	87	80	89	93
28	84	85	80	84	82	75	75
29	104	96	95	90	99	96	92
33	105	95	85	98	98	95	92
35	87	82	82	80	83	91	85
Average	98	94	91	90	91	92	89
Std. Dev.	8.0	8.4	8.0	65	7.2	7.9	6.4
SEM	2.5	2.7	2.5	2.1	2.3	2.5	2.0

Table 14 - Mean Arterial Pressure (mm Hg) for Experimental Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
13	98	91	46	49	50	40	25
19	101	106	61	66	66	68	67
22	100	104	65	68	77	82	86
24	98	96	50	56	55	68	77
27	95	115	46	48	43	28	NA
30	104	102	71	82	82	88	91
31	105	100	50	55	66	65	77
32	80	85	70	54	57	53	50
34	117	125	85	85	80	59	23
36	92	92	50	49	56	71	67
Average	99	102	59	61	63	62	62
Std. Dev.	9.5	11.9	13.2	13.6	13.3	18.3	26.5
SEM	3.0	3.8	4.2	4.3	4.2	5.7	8.8

F. Right Atrial Pressure

Right atrial pressures for control (Table 15) and experimental (Table 16) animals are given, and means and standard errors plotted (Figure 4). Neither group shows significant change over the baseline period from T=0 to T=1 hr. At T=1, control animals had an RAP of 4.4 ± 1.5 mm Hg, compared to 3.4 ± 2.7 mm Hg for experimental animals. These differences were not significant. During the course of the experiment, control animals showed a decrease in RAP which was significant, compared to the control baseline values, for T=7 only, where the mean was 3.7 ± 2.0 mm Hg ($p < 0.05$). At T=2, the experimental animals had an RAP of 1.0 ± 2.9 mm Hg, which varied significantly from control's value of 4.3 ± 1.4 ($p < 0.01$). At T=7 hr, the two groups did not differ significantly, with experimentals being 1.1 ± 3.2 mm Hg.

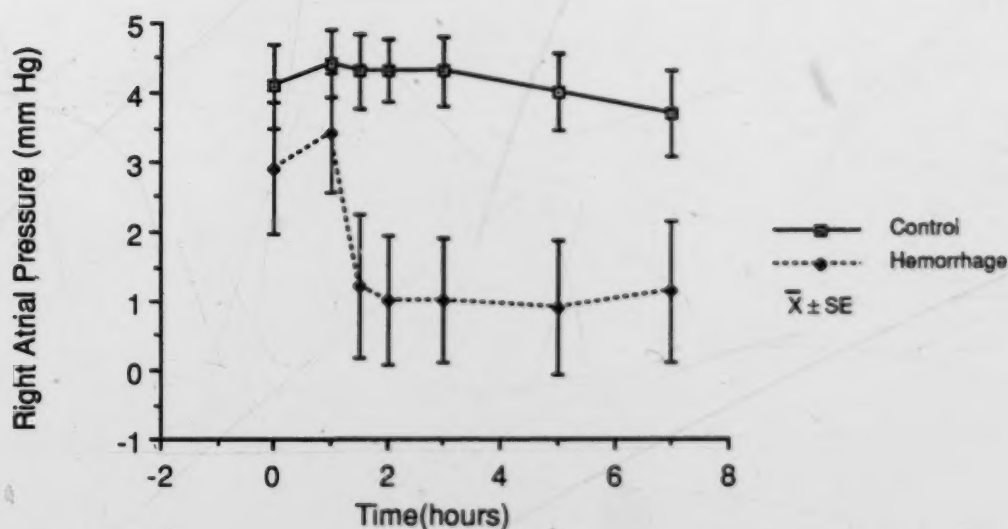


Figure 4 - Right Atrial Pressure For Control and Experimental Animals

Table 15 - Right Atrial Pressures (mm Hg) for Control Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
15	6	4	4	4	3	3	3
17	4	5	5	4	4	4	4
18	7	7	7	7	7	6	6
20	5	5	5	5	6	5	4
23	2	3	3	3	3	2	2
26	5	6	5	5	5	5	5
28	1	2	1	2	2	1	0
29	5	5	6	5	5	6	6
33	4	4	4	5	5	5	5
35	2	3	3	3	3	3	2
Average	4.1	4.4	4.3	4.3	4.3	4.0	3.7
Std. Dev.	1.9	1.5	1.7	1.4	1.6	1.7	2.0
SEM	0.6	0.5	0.5	0.5	0.5	0.5	0.6

Table 16 - Right Atrial Pressure (mm Hg) for Experimental Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
13	7	6	3	3	2	3	4
19	6	6	4	4	NA	NA	NA
22	3	3	0	0	2	1	3
24	5	5	4	4	4	3	3
27	0	5	5	2	3	4	NA
30	0	0	-2	-1	-1	-2	-1
31	4	5	2	2	2	0	2
32	5	5	3	3	3	4	4
34	-2	-1	-5	-5	-5	-5	-5
36	1	0	-2	-2	-1	0	-1
Average	2.9	3.4	1.2	1.0	1.0	0.9	1.1
Std. Dev.	3.0	2.7	3.3	2.9	2.8	3.0	3.2
SEM	1.0	0.9	1.0	0.9	0.9	1.0	1.1

G. Pulmonary Artery Diastolic Pressure

Values for PADP are given for control (Table 17) and experimental (Table 18) animals, and means and standard errors plotted (Figure 5). Neither group showed significant variation over the baseline period. Control animals had a PADP of 10.4 ± 3.2 mm Hg, compared to 8.8 ± 3.2 mm Hg for experimentals at T=1 hr. These differences were not significant. During the experiment, control animals showed no significant variation within the group. At T=2 hr, controls had a PADP of 9.8 ± 2.7 mm Hg, compared to 5.6 ± 3.6 mm Hg for experimental animals ($p < 0.01$). Significant differences were also seen at T=7 hr where controls had a PADP of 10.1 ± 3.0 mm Hg, compared to 4.8 ± 3.0 mm Hg for the experimental group ($p < 0.005$).

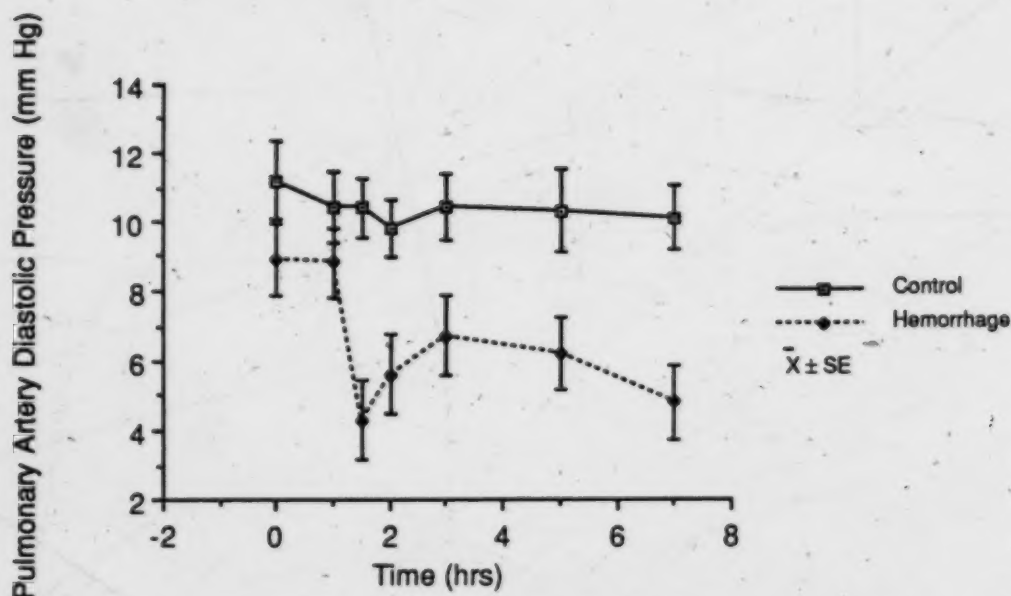


Figure 5 - Pulmonary Artery Diastolic Pressure for Control and Experimental Animals

Table 17 - Pulmonary Artery Diastolic Pressure (mm Hg) for Control Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
15	7	6	7	6	7	6	7
17	17	14	13	13	15	14	10
18	11	9	9	9	9	9	10
20	12	12	12	10	11	11	12
23	8	7	7	7	7	5	8
26	17	16	16	15	14	16	13
28	10	10	10	10	11	9	5
29	11	12	11	11	12	14	15
33	12	11	10	8	12	13	12
35	7	7	9	9	6	6	9
Average	11.2	10.4	10.4	9.8	10.4	10.3	10.1
Std. Dev.	3.6	3.2	2.8	2.7	3.1	3.9	3.0
SEM	1.1	1.0	0.9	0.9	1.0	1.2	1.0

Table 18 - Pulmonary Artery Diastolic Pressure (mm Hg) for Experimental Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
13	13	12	6	6	7	8	6
19	10	10	4	4	NA	NA	NA
22	7	7	3	4	6	6	6
24	11	10	7	8	9	11	7
27	10	10	10	10	6	6	NA
30	4	4	0	0	3	2	0
31	9	9	4	9	11	7	5
32	11	11	9	9	9	9	9
34	11	12	0	6	9	6	4
36	3	3	0	0	0	1	1
Average	8.9	8.8	4.3	5.6	6.7	6.2	4.8
Std. Dev.	3.3	3.2	3.7	3.6	3.4	3.2	3.0
SEM	1.0	1.0	1.2	1.1	1.1	1.1	1.1

H. Pulmonary Wedge Pressure

Pulmonary wedge pressures were recorded for control (Table 19) and experimental (Table 20) animals, and the means and standard errors plotted (Figure 6). Over the baseline period of T=0 and T=1 hrs, the two groups showed no significant change. At T=1 hr, the control animals had a PWP of 5.3 ± 1.8 mm Hg, compared to 4.5 ± 3.3 mm Hg for experimentals, and these differences were not significant. The control group showed no significant variation within group over time. At T=2 hr. the PWP for controls was 5.0 ± 1.3 mm Hg, and for experimental animals 2.2 ± 2.6 mm Hg ($p < 0.05$). At T=7 hr, the differences were not significant, with controls having a PWP of 4.7 ± 2.2 mm Hg, compared to 2.0 ± 3.2 mm Hg for experimental animals.

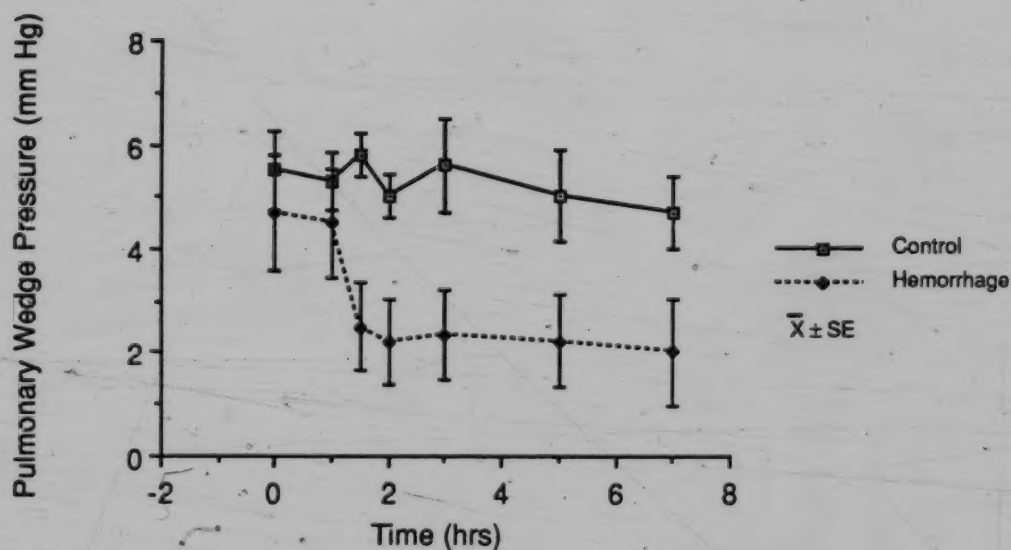


Figure 6 - Pulmonary Wedge Pressure for Control and Experimental Animals

Table 19 - Pulmonary Wedge Pressure (mm Hg) for Control Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
15	6	6	7	6	5	5	4
17	9	5	7	5	12	9	4
18	8	7	7	7	7	7	7
20	7	8	8	7	7	6	7
23	5	5	5	4	4	2	3
26	7	7	5	5	5	6	5
28	2	5	5	5	4	2	0
29	4	4	5	4	5	6	6
33	5	4	5	3	6	7	7
35	2	2	4	4	1	0	4
Average	5.5	5.3	5.8	5.0	5.6	5.0	4.7
Std. Dev.	2.4	1.8	1.3	1.3	2.8	2.8	2.2
SEM	0.8	0.6	0.4	0.4	0.9	0.9	0.7

Table 20 - Pulmonary Wedge Pressure (mm Hg) for Experimental Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
13	8	8	3	3	3	3	5
19	7	8	4	4	NA	NA	NA
22	5	5	2	1	3	3	3
24	8	7	7	5	5	5	5
27	5	5	4	4	3	4	NA
30	1	0	-1	0	0	-1	-1
31	7	6	4	5	6	4	3
32	7	6	4	3	4	5	5
34	-2	0	-1	-3	-2	-3	-3
36	1	0	-1	0	-1	0	-1
Average	4.7	4.5	2.5	2.2	2.3	2.2	2.0
Std. Dev.	3.5	3.3	2.7	2.6	2.7	2.9	3.2
SEM	1.1	1.0	0.9	0.8	0.9	1.0	1.1

I. Cardiac Index

Cardiac index for control (Table 21) and experimental (Table 22) animals are given, and the means and standard errors plotted (Figure 7). In considering variation within the control group, CI decreased significantly with time. At T=1 hr CI was 140 ± 17.7 ml/min/Kg, at T=2 hr it was 130 ± 12.4 ml/min/Kg, and at T=7 it was 108 ± 15.8 ml/min/Kg ($p < 0.05$ and $p < 0.0005$ respectively). Prior to hemorrhage, at T=1 hr, the experimental group had a CI of 146 ± 18.6 ml/min/Kg which was not significantly different from the control value. However, a marked decrease in CI, to 72 ± 14.4 ml/min/Kg at T=2 hr, was observed, and this differed significantly from controls ($p < 0.0005$). At T=7 hr, the experimental group failed to show a significant difference from controls, with a CI of 83 ± 36.1 ml/min/Kg. It should be noted three of ten values were missing in this group.

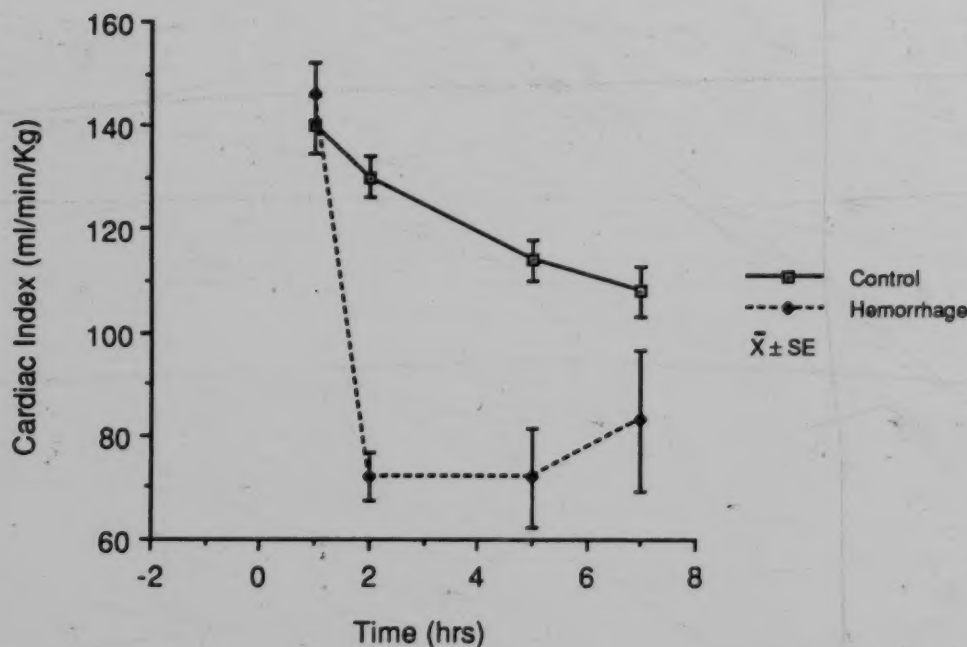


Figure 7 - Cardiac Index for Control and Experimental Animals

Table 21 - Cardiac Index (ml/min/Kg) for Control Animals

Pig #	Time (hrs)			
	T=1	T=2	T=5	T=7
15	146	122	118	103
17	166	138	119	110
18	158	143	128	135
20	148	145	106	97
23	141	140	131	129
26	118	111	96	94
28	111	115	99	93
29	136	132	120	119
33	132	127	109	96
35	104	100	80	78
Average	140	130	114	108
Std. Dev.	17.7	12.4	12.3	15.8
SEM	5.6	3.9	3.9	5.0

Table 22 - Cardiac Index (ml/min/Kg) for Experimental Animals

Pig #	Time (hrs)			
	T=1	T=2	T=5	T=7
13	181	69	41	20
19	174	90	NA	NA
22	152	97	115	119
24	140	66	75	80
27	131	60	48	NA
30	145	77	102	118
31	134	82	85	92
32	136	52	52	51
34	141	61	43	NA
36	123	63	90	98
Average	146	72	72	83
Std. Dev.	18.6	14.4	27.5	36.1
SEM	5.9	4.6	9.2	13.7

J. Lymph Flow

Lymph flow is given for control (Table 23) and experimental animals (Table 24), and means and standard errors plotted (Figure 8). In this group of results, standard deviations were larger than for most others. This data involved collections over an hour period, thus the prebleed values are given by T=0 hr, the first hour postbleed given by T=1 hr, and the final two hr collection given by T=5 hr. Values of LF at T=0 hr, 1.5 ± 1.5 ml/hr for controls and 1.1 ± 0.9 ml/min for experimental animals, did not differ significantly. Lymph flow within the control group did not differ significantly, with rates of 1.4 ± 1.1 and 2.9 ± 1.9 ml/hr for T=1 and 5 hrs respectively. After the first hour of hemorrhage, at T=1 hr, LF increased to 2.6 ± 2.2 ml/hr, but this was not significant. Similarly, LF increased in the hemorrhage group at T=5 hr, to 4.2 ± 3.7 ml/hr, but again this increase was not significant.

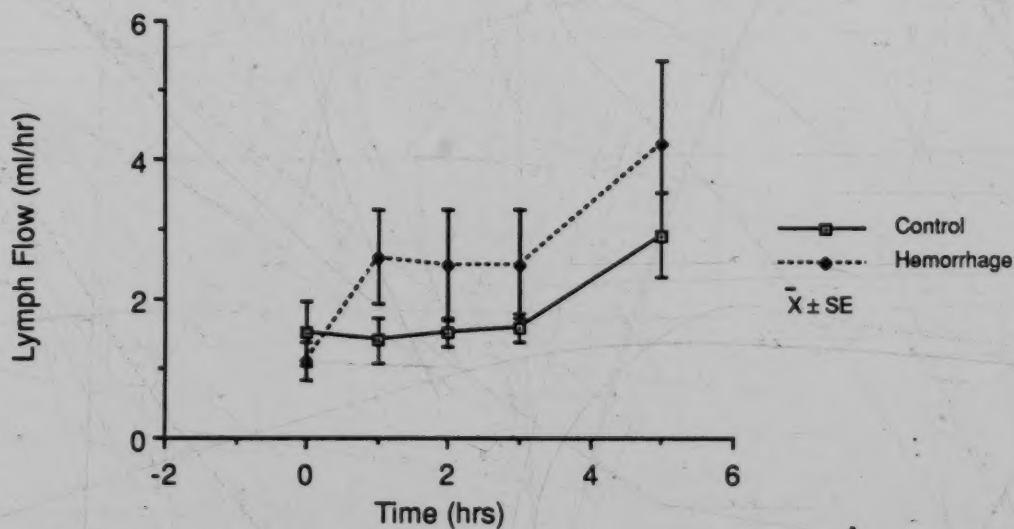


Figure 8 - Lymph Flow for Control and Experimental Animals

Table 23 - Lymph Flow (ml/hr) for Control Animals

Pig #	Time (hrs)				
	T=0	T=1	T=2	T=3	T=5
15	1.7	1.2	1.8	1.9	3.6
17	0.9	1.3	1.8	1.9	1.8
18	1.2	3.9	2.1	2.2	3.4
20	1.0	1.0	1.6	2.5	3.3
23	0.6	0.8	1.0	1.1	1.6
26	0.4	0.2	0.3	0.3	0.4
28	0.9	2.6	2.5	2.0	1.1
29	0.6	0.7	1.6	1.2	1.8
33	2.3	1.5	1.3	1.1	6.6
35	5.5	1.2	1.2	2.0	5.0
Average	1.5	1.4	1.5	1.6	2.9
Std. Dev.	1.5	1.1	0.6	0.7	1.9
SEM	0.5	0.3	0.2	0.2	0.6

Table 24 - Lymph Flow (ml/min) for Experimental Animals

Pig #	Time (hrs)				
	T=0	T=1	T=2	T=3	T=5
13	1.6	2.8	2.0	1.9	2.1
19	0.9	1.2	0.9	1.5	6.3
22	0.4	0.5	0.9	1.5	2.5
24	0.3	0.5	0.3	1.1	2.2
27	2.8	4.4	3.0	2.0	NA
30	2.0	2.7	2.4	3.4	7.8
31	0.8	1.4	1.2	2.3	3.2
32	0.4	1.0	7.0	0.7	1.0
34	0.4	7.0	0.4	1.0	1.1
36	1.7	4.7	6.7	9.2	11.8
Average	1.1	2.6	2.5	2.5	4.2
Std. Dev.	0.9	2.2	2.5	2.5	3.7
SEM	0.3	0.7	0.8	0.8	1.2

K. Craniomesenteric Arterial Flow

Craniomesenteric arterial flow was recorded for control (Table 25) and experimental (Table 26) animals, and the means and standard errors plotted (Figure 9). Both groups showed no significant variation over the baseline period. At T=1 hr, the control animals had a CAF of 7.9 ± 1.8 ml/min/Kg, compared to 10.2 ± 2.7 ml/min/Kg for the experimental animals. These differences were not significant. The control group showed no significant variation over time, with values of 8.2 ± 1.7 and 7.9 ± 1.8 ml/min/Kg at T=2 and 7 hrs respectively. In contrast, the experimental group showed a marked drop in CAF at T=2 hr, to 6.6 ± 2.2 ml/min/Kg, and this was significant ($p < 0.05$). By T=7 hr, the CAF in these animals had returned towards prebleed levels, with a mean of 7.9 ± 4.0 ml/min/Kg, and was no longer significantly different from controls.

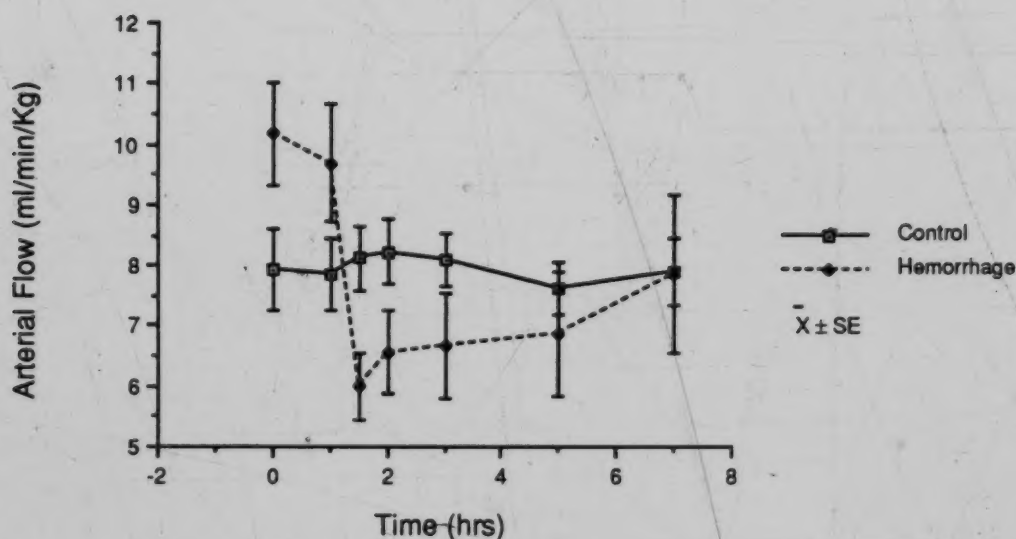


Figure 9 - Craniomesenteric Arterial Flow For Control and Experimental Animals

Table 25 - Craniomesenteric Arterial Flow (ml/min/Kg) for Control Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
15	5.4	5.2	5.7	5.7	6.2	5.6	5.5
17	5.7	6.6	7.1	7.0	7.3	7.2	7.0
18	7.5	8.5	9.5	9.1	9.1	9.1	10.3
20	6.3	6.8	7.2	8.8	8.0	7.7	7.6
23	7.2	7.2	7.5	7.5	7.2	6.9	6.9
26	8.0	6.8	7.4	7.2	7.2	7.7	7.6
28	10.5	10.0	9.5	9.5	8.4	7.8	8.8
29	6.9	6.7	6.3	6.3	6.9	5.6	5.6
33	10.4	10.3	10.6	10.8	10.4	9.6	10.7
35	11.4	10.4	10.3	10.3	10.0	8.9	8.8
Average	7.9	7.9	8.1	8.2	8.1	7.6	7.9
Std. Dev.	2.1	1.8	1.7	1.7	1.4	1.4	1.8
SEM	0.7	0.6	0.5	0.5	0.4	0.4	0.6

Table 26 - Craniomesenteric Arterial Flow (ml/min/Kg) for Experimental Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1.5	T=2	T=3	T=5	T=7
13	11.4	12.4	5.3	5.4	5.0	3.2	1.0
19	12.0	12.5	6.5	10.0	11.8	12.2	12.9
22	9.3	8.5	6.9	8.1	8.2	9.3	10.4
24	10.4	10.2	7.1	7.5	8.0	9.8	10.0
27	13.8	14.2	5.0	4.4	2.7	2.6	NA
30	11.8	10.6	6.9	7.2	8.0	10.0	10.5
31	6.0	5.7	4.5	4.9	5.7	6.9	8.0
32	8.9	9.3	6.9	6.4	5.9	5.4	6.1
4	12.5	9.5	8.5	8.9	8.2	5.1	2.4
36	5.7	4.0	2.2	2.7	3.2	4.2	9.5
Average	10.2	9.7	6.0	6.6	6.7	6.9	7.9
Std. Dev.	2.7	3.1	1.8	2.2	2.7	3.3	4.0
SEM	0.9	1.0	0.6	0.7	0.9	1.0	1.3

L. Urine Output

Urine output for control (Table 27) and experimental (Table 28) animals is given, and the means and standard errors plotted (Figure 10). As with lymph flow, the results given represent collections over time. The results of urine output up to T=0 hr have already been given (*vide supra*). Control animals had a significantly lower output of 1.4 ± 0.5 ml/hr/Kg at T=0 hr, compared to 2.4 ± 1.5 ml/hr/Kg for experimentals ($p < 0.05$). Controls showed no significant variation within the group, with values of 1.5 ± 0.6 and 1.2 ± 0.5 ml/hr/Kg for T=1 and 5 hrs respectively. The experimental animals showed significant decreases in urine output after hemorrhage, with values of 0.7 ± 0.5 and 0.4 ± 0.3 ml/hr/Kg for T=1 and 5 hrs respectively ($p < 0.005$ and $p < 0.0005$).

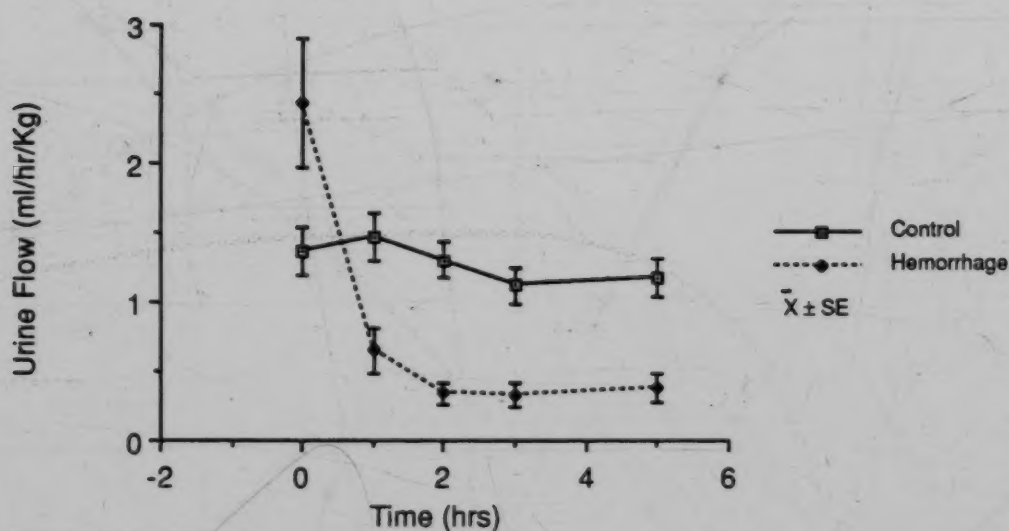


Figure 10 - Urine Output for Control and Experimental Animals

Table 27 - Urine Output/Body Weight (ml/hr/Kg) for Control Animals

Pig #	Time (hrs)				
	T=0	T=1	T=2	T=3	T=5
15	0.5	1.0	0.8	0.8	0.6
17	0.8	0.8	0.8	0.8	0.9
18	1.6	1.5	1.6	1.1	1.0
20	2.2	1.6	1.6	2.1	1.0
23	1.3	1.5	1.2	0.9	1.0
26	1.1	1.5	2.0	1.4	1.5
28	2.0	1.1	1.1	1.0	1.0
29	1.6	2.3	1.7	1.3	1.3
33	1.5	2.4	1.3	1.3	2.2
35	1.0	1.0	1.0	0.6	1.4
Average	1.4	1.5	1.3	1.1	1.2
Std. Dev.	0.5	0.6	0.4	0.4	0.5
SEM	0.2	0.2	0.1	0.1	0.1

Table 28 - Urine Output/Body Weight (ml/hr/Kg) for Experimental Animals

Pig #	Time (hrs)				
	T=0	T=1	T=2	T=3	T=5
13	2.1	0.6	0.2	0.1	0.1
19	2.1	0.7	0.7	0.7	0.7
22	1.2	0.6	0.7	0.7	0.8
24	2.3	0.4	0.2	0.2	0.4
27	5.8	1.8	0.0	0.0	NA
30	1.0	0.4	0.6	0.6	0.8
31	4.1	0.2	0.2	0.5	0.6
32	1.1	0.2	0.0	0.0	0.0
34	2.0	0.6	0.5	0.2	0.0
36	2.7	1.2	0.5	0.4	0.6
Average	2.4	0.7	0.3	0.3	0.4
Std. Dev.	1.5	0.5	0.3	0.3	0.3
SEM	0.5	0.2	0.1	0.1	0.1

M. Temperature

Core temperatures were recorded for control (Table 29) and experimental (Table 30) animals. Control animals showed a significant increase in temperature from T=0 to T=1 hrs, with values of 38.1 ± 0.6 and 38.7 ± 0.2 °C ($p < 0.005$). The temperature for controls at T=2 hr, 38.7 ± 0.3 °C, was not significantly different, but at T=7 hr there was significant increase, to 39.0 ± 0.4 °C ($p < 0.05$). The experimental animals did not show a

significant increase over the baseline period, with a temperature of 38.7 ± 0.4 °C at T=1 hr. This did not differ significantly from controls. However, the temperatures for experimental animals at T=2 and T=7 hrs, 39.2 ± 0.4 and 39.9 ± 0.6 °C, did differ significantly from controls ($p < 0.005$ and $p < 0.0005$ respectively).

Table 29 - Core Temperature (°C) for Control Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1	T=2	T=3	T=5	T=7
15	37.3	38.3	38.7	38.7	38.6	38.7	38.9
17	38.0	38.5	38.3	38.1	38.8	38.6	38.8
18	37.5	38.4	38.8	38.8	38.7	38.8	38.6
20	37.5	38.6	39.0	39.0	38.5	38.5	38.5
23	38.1	39.0	39.1	38.9	38.6	38.8	39.0
26	38.8	38.8	39.0	39.1	39.2	39.8	40.0
28	38.5	38.7	38.5	38.7	38.8	38.6	38.8
29	38.8	38.7	38.6	38.5	38.8	38.8	38.8
33	38.3	38.8	38.5	38.6	38.7	39.3	38.9
35	38.6	38.8	38.8	38.7	38.7	39.0	39.2
Average	38.1	38.7	38.7	38.7	38.7	38.9	39.0
Std. Dev.	0.6	0.2	0.3	0.3	0.2	0.4	0.4

Table 30- Core Temperature (°C) for Experimental Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1	T=2	T=3	T=5	T=7
13	37.5	38.1	38.6	38.6	38.9	39.3	38.9
19	38.4	38.7	39.0	39.1	39.0	39.0	39.5
22	38.6	39.0	39.1	39.1	39.3	40.0	40.4
24	39.8	39.5	39.2	39.3	39.4	40.3	40.6
27	38.6	38.8	39.5	39.8	40.2	39.6	NA
30	38.7	38.7	38.8	38.8	39.4	39.5	39.8
31	38.7	38.8	38.9	38.9	39.1	39.7	40.0
32	38.7	38.7	39.1	39.2	39.3	39.5	39.5
34	37.1	38.1	39.0	39.5	40.0	40.8	40.8
6	38.8	38.9	39.4	39.4	39.4	39.9	40.0
Average	38.5	38.7	39.1	39.2	39.4	39.8	39.9
Std. Dev.	0.7	0.4	0.3	0.4	0.4	0.5	0.6

N. Respiratory Rate

Respiratory rates for control (Table 31) and experimental (Table 32) animals are given. Control and experimental animals showed no significant variation over the baseline period, with RRs of 17 ± 1.3 and 16 ± 1.1 b/min at T=1 hr. Control animals showed little variation within group except for T=7 hr, where the rate of 18 ± 0.9 b/min did differ significantly from the T=1 hr value ($p < 0.05$). Experimental animals showed some change in RR with time, with values of 16 ± 1.1 and 17 ± 1.0 b/min at T=2 and T=7 hrs, however these were not significantly different from controls.

Table 31 - Respiratory Rate (b/min) for Control Animals

Pig #	Time (hrs)						
	T=0	T=1	T=1	T=2	T=3	T=5	T=7
15	15	15	15	16	15	18	18
17	16	16	16	16	16	17	18
18	17	17	18	18	18	20	18
20	16	16	16	16	16	17	17
23	18	18	18	16	16	18	18
26	18	18	18	18	18	18	18
28	16	16	16	16	16	16	16
29	16	18	16	16	18	18	18
33	16	15	16	16	16	16	16
35	18	18	18	18	18	18	18
Average	17	17	17	17	17	18	18
Std. Dev.	1.1	1.3	1.2	1.0	1.2	1.2	0.9

Table 32 - Respiratory Rate (b/min) for Experimental Animals

Fig #	Time (hrs)						
	T=0	T=1	T=1	T=2	T=3	T=5	T=7
13	15	16	16	16	18	18	18
19	16	18	18	18	18	18	18
22	16	16	16	16	16	16	16
24	16	16	16	16	16	16	16
27	16	16	16	16	16	15	NA
30	16	16	16	16	16	16	16
31	18	18	18	18	18	18	18
32	16	16	16	16	17	15	16
34	16	16	16	16	16	16	16
36	14	14	16	14	14	16	16
Average	16	16	16	16	17	16	17
Std. Dev.	1.0	1.1	0.8	1.1	1.3	1.2	1.0

IV. Biochemistry Results

A. Arterial Partial Pressure of Oxygen

The P_{aO_2} for control (Table 33) and experimental (Table 34) animals are given. Control animals showed no significant variation within the group, nor did experimental animals. The P_{aO_2} for controls was consistently but not significantly lower than that of experimental animals. At T=1, P_{aO_2} for controls was 192.4 ± 87.1 mm Hg, compared to 211.8 ± 87.5 mm Hg for experimental animals. At T=2, control animals had a P_{aO_2} of 193.6 ± 84.3 mm Hg, versus 223.2 ± 85.1 mm Hg for experimentals. At T=7, controls had a P_{aO_2} of 175.9 ± 66.5 mm Hg, and experimentals a P_{aO_2} of 215.4 ± 98.9 mm Hg.

Table 33 - Partial Pressure of Oxygen (mm Hg) of Arterial Blood for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	416.0	421.0	420.0	450.0	452.0	340.0
17	122.7	118.6	116.9	130.8	129.6	116.1
18	209.0	205.4	159.9	158.7	159.4	168.1
20	180.2	174.5	170.3	178.5	165.3	163.6
23	279.0	213.5	220.2	201.9	197.4	190.7
26	161.0	163.4	163.6	169.3	159.5	166.0
28	173.4	173.5	167.3	170.8	175.7	175.4
29	128.5	190.5	201.1	184.6	181.3	204.0
33	175.1	154.6	169.8	167.6	154.9	141.0
35	123.1	108.6	146.5	181.1	180.9	93.7
Average	196.8	192.4	193.6	199.3	195.6	175.9
Std. Dev.	90.1	87.1	84.3	90.0	92.0	66.5

Table 34 - Partial Pressure of Oxygen (mm Hg) of Arterial Blood for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	381.0	457.0	455.0	471.0	455.0	436.0
19	165.1	165.6	178.7	158.7	168.9	160.4
22	188.2	183.6	182.4	180.3	169.2	168.7
24	302.0	196.9	222.3	199.4	185.4	124.8
27	186.7	189.1	190.0	176.6	207.8	NA
30	139.2	187.3	204.1	234.2	187.2	268.3
31	200.2	203.5	178.8	196.3	198.4	216.4
32	212.8	202.4	215.3	205.2	234	162.7
34	208.8	155.2	244.4	182.5	96.2	NA
36	179.8	177.6	161.3	163.8	174	186.1
Average	216.4	211.8	223.2	216.8	207.6	215.4
Std. Dev.	71.8	87.5	85.1	92.0	93.9	98.9

B. Arterial Partial Pressure of Carbon Dioxide

The P_aCO_2 for control (Table 35) and experimental (Table 36) animals are given. Control animals showed no significant variation within the group over time, with values of 40.9 ± 3.0 , 43.9 ± 3.0 , and 40.5 ± 0.4 mm Hg at times T=1, 2, and 7 respectively. The corresponding values for experimental animals were 41.2 ± 2.8 , 43.1 ± 2.5 , and 40.8 ± 4.2 mm Hg, and these did not differ significantly.

Table 35 - Partial Pressure of Carbon Dioxide (mm Hg) of Arterial Blood for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	44.5	46.7	46.5	48.3	45.6	40.4
17	45.1	43.8	40.6	39.9	42.0	40.8
18	45.0	46.1	46.1	44.4	44.3	44.3
20	43.8	45.4	46.6	46.4	45.4	49.5
23	43.3	42.3	40.9	41.2	42.5	46.1
26	45.0	45.1	42.4	39.8	42.0	39.3
28	43.3	42.6	44.9	42.0	41.7	41.1
29	34.7	43.5	43.2	47.5	43.2	45.5
33	40.7	37.9	41.7	42.7	45.6	40.2
35	43.8	42.1	46	44.3	42.7	40.7
Average	42.3	40.0	43.9	43.5	44.2	40.5
Std. Dev.	2.2	3.0	3.0	1.1	2.1	0.4

Table 36 - Partial Pressure of Carbon Dioxide (mm Hg) of Arterial Blood for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	45.9	45	46.8	48.3	48.8	32.4
19	44.7	43.8	46.6	47.2	44.7	43.1
22	43.7	42.4	46	40.6	42.9	40.5
24	41.4	36.5	40.6	41.1	41.1	41.1
27	42.5	43.6	42.7	46.0	43.3	NA
30	40.7	41.6	40.2	42.4	45.6	44.5
31	42.8	40.5	41.0	42.2	42.8	46.2
32	37.8	41.7	42.6	45.9	43.1	40.0
34	40.0	37.8	42.9	42.6	42.1	NA
36	39.4	38.9	41.2	43.3	43.7	38.9
Average	41.9	41.2	43.1	44.0	43.8	40.8
Std. Dev.	2.5	2.8	2.5	2.7	2.2	4.2

C. Hydrogen Ion Concentration

The arterial $[H^+]$ for control (Table 37) and experimental (Table 38) animals are given. Control animals show little variation over time, with $[H^+]$ s of 39.7 ± 2.8 , 39.0 ± 3.2 , and 39.0 ± 2.0 nmol/L at T=1, 2 and 7 respectively. The experimental animals showed little variation over the baseline period, with an $[H^+]$ of 38.1 ± 5.4 nmol/L at T=1, and this did not differ significantly from controls. At T=2 however, they showed a significant increase in $[H^+]$, to 43.9 ± 4.3 nmol/L, and this differed significantly from controls ($p < 0.05$). Hydrogen ion concentration for experimental animals increased during the next several hours, but by T=7 hr, $[H^+]$ for experimental animals had returned towards normal, with a value of 40.6 ± 5.0 nmol/L, and this difference was not significant.

Table 37 - Hydrogen Ion Concentration (nmol/L) of Arterial Blood for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	39	43	43	45	45	43
17	42	38	40	38	39	39
18	36	40	37	39	36	39
20	42	40	43	43	41	39
23	36	34	34	32	36	40
26	43	43	42	38	36	37
28	34	42	35	39	40	40
29	43	39	39	44	41	37
33	39	41	40	40	42	40
35	38	37	37	36	38	36
Average	39.2	39.7	39.0	39.4	39.4	39.0
Std. Dev.	3.2	2.8	3.2	3.9	3.0	2.0

Table 38 - Hydrogen Ion Concentration (nmol/L) of Arterial Blood for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	42	44	48	52	58	50
19	41	44	47	47	43	44
22	40	39	43	40	44	37
24	34	30	43	43	40	38
27	42	47	51	59	69	NA
30	32	33	36	40	42	37
31	39	37	40	41	42	44
32	35	37	46	48	46	35
34	40	35	44	37	54	NA
36	36	35	41	43	43	40
Average	38.1	38.1	43.9	45.0	48.1	40.6
Std. Dev.	3.6	5.4	4.3	6.6	9.3	5.0

D. Arterial Bicarbonate Concentration

The HCO_3^- for control (Table 39) and experimental (Table 40) animals are given. Control animals showed little variation in HCO_3^- , with values of 26.2 ± 2.2 , 26.8 ± 2.3 , and 26.2 ± 2.6 mmol/L for times T=1, 2, and 7 respectively. Experimental animals showed little variation over the baseline period, and had a HCO_3^- of 25.7 ± 2.4 mmol/L at T=1 which did not differ significantly from control values. At T=2, however, the experimental group showed a significant decrease, to 23.4 ± 1.8 mmol/L ($p < 0.005$). As

for $[H^+]$, the HCO_3 for experimental animals continued to decrease. However at $T=7$ the HCO_3 , 23.4 ± 3.9 mmol/L, was not significantly different from controls.

Table 39 - Arterial Bicarbonate (mmol/L) for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	26.7	25.9	25.9	25.3	24.1	22.2
17	25.7	27.4	24.1	24.8	25.8	25.0
18	29.0	27.2	29.5	27.2	28.8	27.2
20	24.9	27.1	25.6	25.9	26.4	30.2
23	29.4	30.2	29.0	30.7	29.1	28.7
26	25.0	25.2	24.1	24.9	27.0	24.9
28	29.5	23.9	29.5	25.3	25.0	24.3
29	19.2	26.5	26.4	25.9	25.1	28.7
33	24.8	22.1	24.8	25.0	26.1	23.7
35	27.2	27.1	29.3	28.9	26.7	26.6
Average	26.1	26.3	26.8	26.4	26.4	26.2
Std. Dev.	3.1	2.2	2.3	2.0	1.6	2.6

Table 40 - Arterial Bicarbonate (mmol/L) for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	26.5	24.2	23.0	21.8	20.0	15.4
19	26.1	23.7	23.3	23.6	25.0	23.1
22	25.8	25.5	25.5	24.4	23.3	25.4
24	29.1	29.2	22.7	22.7	24.3	25.3
27	24.0	21.8	19.8	18.6	14.9	NA
30	29.8	29.7	26.4	25.0	26.1	28.5
31	26.1	25.6	24.4	24.2	24.4	25.3
32	25.4	26.4	22.0	22.7	22.0	21.3
34	24.0	25.0	23.1	21.5	18.5	NA
36	25.7	25.6	24.0	23.8	24.2	23.2
Average	26.3	25.7	23.4	22.8	22.3	23.4
Std. Dev.	1.9	2.4	1.8	1.9	3.5	3.9

E. Mixed Venous Oxygen

The P_{vO_2} for control (Table 41) and experimental (Table 42) animals are given. The control animals showed little variation over the baseline period, and then a steady decrease in P_{vO_2} over time, with values of 46.7 ± 5.0 , 45.8 ± 3.8 , and 43.0 ± 5.0 mm Hg

at times T=1, 2, and 7 respectively. These differences were not significant. Experimental animals showed little baseline variation, with a P_vO_2 of 47.7 ± 7.5 mm Hg at T=1. At T=2, the experimental animals measured 34.4 ± 9.6 mm Hg, and this was a significant decrease compared to controls ($p < 0.005$). By T=7, the experimental group had a P_vO_2 of 37.2 ± 14.8 mm Hg, and this did not differ significantly from controls. However, two points worth noting are that there were only six data points for this time period for experimental animals, and the results for Pig # 13 were exceptionally high, probably due to the high inspired oxygen concentration (due to a faulty gas blender).

Table 41 - Partial Pressure of Oxygen (mm Hg) of Mixed Venous Blood for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	46.3	45.9	45.1	45.0	41.6	44.6
17	50.8	43.8	44.8	45.5	48.1	49.5
18	50.3	56.9	52.2	50.7	45.7	41.1
20	47.6	44.8	48.8	44.0	40.3	41.9
23	52.9	48.5	46.1	45.9	47.7	49.9
26	45.8	46.1	44.0	42.5	41.1	40.5
28	42.8	43.2	41.9	42.1	32.8	44.5
29	48.4	49.9	49.2	50.4	47.2	46.3
33	50.1	49.4	46.8	46.6	46.8	41.9
35	39.4	38.1	39.1	38.3	36.7	32.6
Average	47.4	46.7	45.8	45.1	42.8	43.0
Std. Dev.	4.1	5.0	3.8	3.8	5.2	5.0

Table 42 - Partial Pressure of Oxygen (mm Hg) of Mixed Venous Blood for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	54.8	48.7	57.1	62.4	69.4	64.8
19	42.2	42.2	29.2	NA	NA	NA
22	49.2	49.0	37.2	34.3	36.7	NA
24	44.6	39.8	21.6	19.1	21.7	20.9
27	48.7	51.9	29.7	32.5	29.8	NA
30	32.0	43.5	38.3	34.9	42.2	35.1
31	47.8	48.8	37.1	33.5	35.0	38.5
32	43.7	45.8	30.2	31.9	28.9	31.2
34	55.1	65.8	36.1	28.8	25.6	NA
36	41.4	41.6	27.5	28.7	31.9	32.7
Average	46.0	47.7	34.4	34.0	35.7	37.2
Std. Dev.	6.8	7.5	9.6	11.7	14.0	14.8

F. Hemoglobin

The *Hg* for control (Table 43) and experimental (Table 44) animals are given. Control animals showed little variation over the baseline period, with a *Hg* of 9.5 ± 0.5 g/dl at T=1. This group did show an increase over time, with values of 11.0 ± 1.4 and 13.0 ± 1.4 g/dl at T=2 and 7 respectively, however these did not differ significantly from the baseline measurements. The experimental group also showed little variation over the baseline period, with a *Hg* of 11.3 ± 1.8 g/dl at T=1. Neither this, or the results at T=2, 11.7 ± 1.5 differed significantly from controls. There was however a gradual decrease in the *Hg* for the experimental group, to 10.6 ± 1.5 g/dl at T=7, and this was significant ($p < 0.05$).

Table 43 - Hemoglobin (g/dl) for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	11.0	9.8	10.1	10.1	10.8	11.8
17	11.9	11.2	11.4	12.0	13.7	12.4
18	8.9	8.4	8.9	8.6	10.4	9.9
20	9.8	10.4	10.8	10.8	11.6	12.1
23	10.2	10.5	10.4	11.5	13.0	13.2
26	11.9	12.6	13.5	13.5	13.4	12.0
28	12.4	13.2	13.1	13.3	13.6	14.0
29	12.7	12.9	13.4	14.3	14.6	14.6
33	11.7	9.1	12.0	12.5	13.5	13.7
35	9.7	9.8	10.0	10.3	NA	NA
Average	10.7	9.5	11.0	11.4	12.7	13.0
Std. Dev.	1.4	0.5	1.4	1.6	1.5	1.4

Table 44 - Hemoglobin (g/dl) for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	10.6	10.8	11.3	11.7	11.1	11.0
19	8.0	7.9	8.8	8.0	7.9	7.8
22	9.3	9.3	10.3	10.1	10.6	9.9
24	12.1	12.1	11.8	11.2	11.7	11.2
27	13.6	13.1	13.0	11.7	10.2	NA
30	10.4	10.4	11.4	11.4	11.4	10.4
31	13.3	14.0	13.1	14.0	13.0	10.6
32	11.7	11.7	13.5	14.0	13.4	13.0
34	11.4	11.6	11.3	11.3	11.1	NA
36	10.7	11.9	12.8	12.1	12.1	11.2
Average	11.1	11.3	11.7	11.6	11.3	10.6
Std. Dev.	1.7	1.8	1.5	1.7	1.5	1.5

G. Hematocrit

The Hct for control (Table 45) and experimental (Table 46) animals are given. Controls showed a significant variation over the baseline period, with a Hct of 33.7 ± 4.1 % ($p < 0.05$) at T=1. The results at T=2, 34.0 ± 3.8 % did not differ significantly, though those at T=7, 36.5 ± 5.2 %, did ($p < 0.05$). In contrast, the hemorrhage group showed little variation early on, with Hcts of 33.5 ± 6.0 , and 34.5 ± 4.6 % at T=1 and 2 respectively. By T=7 however, the experimental group showed a significant decrease to 31.4 ± 4.7 %, when compared to controls ($p < 0.05$).

Table 45 - Hematocrit (%) for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	30	31	31	31	32	34
17	35	37	36	36	39	37
18	27	30	28	28	27	26
20	30	31	32	33	35	35
23	30	34	34	34	38	39
26	35	38	37	37	40	38
28	34	39	39	37	35	40
29	37	38	39	39	43	45
33	33	32	34	36	34	39
35	27	27	30	31	30	32
Average	31.8	33.7	34.0	34.2	35.3	36.5
Std. Dev.	3.5	4.1	3.8	3.4	4.9	5.2

Table 46 - Hematocrit (%) for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	31	32	34	34	33	32
19	24	24	25	24	24	23
22	28	27	29	29	31	27
24	34	35	34	32	32	32
27	37	38	38	35	30	NA
30	31	31	37	34	34	31
31	37	46	37	38	36	37
32	34	34	40	43	40	37
34	32	34	33	33	32	NA
36	33	34	37.5	34	33	32
Average	32.1	33.5	34.5	33.6	32.5	31.4
Std. Dev.	4.0	6.0	4.6	5.0	4.1	4.7

H. Serum Total Protein

The TP of serum for control (Table 47) and experimental (Table 48) animals are given. Control animals showed little variation over time, with values of 4.42 ± 0.42 , 4.59 ± 0.41 and 4.69 ± 0.38 g/dl, at times T=1, 2 and 7 respectively. Experimental animals showed little variation over the baseline period with a TP of 4.62 ± 0.29 g/dl at T=1, and this did not differ significantly from controls. At T=2, the experimental group had a TP of

4.42 $\pm$ 0.16 g/dl, which though decreased, did not differ significantly from controls. By T=7, the experimentals had a TP of 4.25 $\pm$ 0.49, and this did differ significantly from controls ($p < 0.05$).

Table 47 - Serum Total Protein (g/dl) for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	4.47	4.71	4.77	4.76	5.02	4.74
17	4.69	4.60	4.61	4.31	4.71	4.79
18	4.35	4.21	4.81	3.92	4.39	4.88
20	4.19	4.74	4.52	4.64	4.70	4.62
23	4.34	4.39	4.08	4.66	4.66	4.67
26	4.54	4.73	5.03	5.02	4.81	4.47
28	3.92	4.13	3.89	3.65	3.64	3.74
29	4.70	4.84	5.13	4.49	4.53	4.92
33	4.78	3.43	4.81	4.81	5.12	5.12
35	4.25	4.37	4.21	4.66	4.35	4.93
Average	4.42	4.42	4.59	4.49	4.59	4.69
Std. Dev.	0.27	0.42	0.41	0.42	0.41	0.38

Table 48 - Serum Total Protein (g/dl) for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	4.55	4.39	4.16	4.14	3.65	3.14
19	4.36	4.46	4.18	4.25	4.46	4.36
22	5.02	4.76	4.54	4.82	4.83	4.65
24	4.75	4.60	4.49	4.03	4.21	4.35
27	4.50	4.23	4.33	3.68	3.29	NA
30	4.96	4.40	4.35	4.36	4.31	4.24
31	5.21	5.09	4.65	4.47	4.50	4.71
32	4.50	4.4	4.49	4.54	4.39	4.44
34	4.79	4.99	4.57	4.65	4.64	NA
36	4.48	4.84	4.39	4.34	4.3	4.07
Average	4.71	4.62	4.42	4.33	4.26	4.25
Std. Dev.	0.28	0.29	0.16	0.33	0.46	0.49

I. Lymph Total Protein

The TP of lymph for control (Table 50) and experimental (Table 51) animals are given. In this group, samples were collected over an hour period and T=0 represents the

baseline measurement. The control group had TPs of 2.51 ± 0.81 , 2.92 ± 0.70 and 2.60 ± 0.70 g/dl at times T=0, 1 and 5, and these did not differ significantly. Similarly, the hemorrhage group showed little variation over time, with lymph TP measuring 3.20 ± 0.36 , 3.18 ± 0.76 , and 2.72 ± 0.69 g/dl at times T=0, 1, and 5 hrs respectively. These did not differ significantly from controls.

Table 50 - Lymph Total Protein (g/dl) for Control Animals

Pig #	Time (hrs)				
	T=0	T=1	T=2	T=3	T=5
15	2.03	2.31	1.98	2.57	2.63
17	3.34	3.30	3.26	3.27	3.75
18	1.84	1.93	1.93	1.71	1.76
20	2.51	3.09	3.33	2.98	2.97
23	2.81	3.21	3.68	3.75	3.57
26	2.26	NA	2.25	2.41	2.28
28	2.58	2.93	3.69	3.25	3.09
29	3.77	3.69	3.60	3.07	3.41
33	2.54	2.91	2.93	3.16	1.94
35	0.98	2.28	2.49	1.74	1.78
Average	2.51	2.92	3.02	2.76	2.60
Std. Dev.	0.81	0.70	0.62	0.67	0.70

Table 51 - Lymph Total Protein (g/dl) for Experimental Animals

Pig #	Time (hrs)				
	T=0	T=1	T=2	T=3	T=5
13	2.87	2.33	2.97	3.02	3.39
19	3.72	3.67	3.25	2.88	2.07
22	2.83	3.00	3.75	3.46	3.27
24	2.67	2.75	3.10	3.01	2.95
27	2.89	2.88	2.84	2.92	NA
30	3.57	3.91	3.18	3.25	2.27
31	3.30	4.47	4.33	3.47	3.32
32	3.27	3.39	3.20	3.30	3.03
34	3.40	3.51	3.47	3.29	2.83
36	3.49	1.93	1.52	1.31	1.35
Average	3.20	3.18	3.16	2.99	2.72
Std. Dev.	0.36	0.76	0.72	0.63	0.69

J. Serum Albumin

The results of serum albumin measurements for control (Table 52) and experimental (Table 53) animals are given. Serum albumins in control animals were 1.46 ± 0.21 , 1.68 ± 0.18 and 1.86 ± 0.14 g/dl at times T=1, 2 and 7, and variation was not significant compared to baseline. Experimental animals showed little variation over the baseline period, with a mean albumin of 1.91 ± 0.23 g/dl at T=1. Subsequently, there was a steady decrease in the albumin, to 1.79 ± 0.19 and 1.70 ± 0.22 g/dl at T=2 and 7. These differences were not significant when compared to the control group.

Table 52 - Serum Albumin (g/dl) for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	1.80	1.84	2.06	1.95	2.35	1.86
17	1.73	1.67	1.84	1.82	1.76	1.73
18	1.33	1.27	1.43	1.21	1.37	1.45
20	1.48	1.62	1.60	1.56	1.69	1.64
23	1.57	1.57	1.41	1.60	1.58	1.62
26	1.53	1.55	1.7	1.64	1.67	1.49
28	1.56	1.55	1.49	1.44	1.48	1.50
29	2.09	2.25	2.15	1.95	2.03	2.12
33	1.83	1.31	1.80	2.72	1.93	1.96
35	1.59	1.61	1.55	1.64	1.65	1.76
Average	1.71	1.46	1.68	2.18	1.79	1.86
Std. Dev.	0.17	0.21	0.18	0.76	0.20	0.14

Table 53 - Serum Albumin (g/dl) for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	1.84	1.79	1.58	1.65	1.47	1.22
19	1.94	1.66	1.63	1.71	1.93	1.67
22	1.84	1.69	1.59	1.71	1.75	1.69
24	2.04	2.12	1.89	1.80	1.92	1.92
27	1.90	1.81	1.76	1.60	1.41	NA
30	2.05	1.88	1.85	1.79	1.79	1.76
31	1.83	1.78	1.66	1.65	1.62	1.67
32	1.88	1.80	1.74	1.79	1.76	1.75
34	2.17	2.23	2.13	2.01	2.09	NA
36	2.11	2.29	2.03	1.98	2.06	1.94
Average	1.96	1.91	1.79	1.77	1.78	1.70
Std. Dev.	0.12	0.23	0.19	0.14	0.23	0.22

K. Lymph Albumin

The concentrations of albumin of lymph for control (Table 54) and experimental (Table 55) animals are given. As for other lymph samples, collections were over a one hour period. In the control group, there was a significant increase from a baseline value of 1.01 ± 0.40 g/dl at T=0, to 1.18 ± 0.32 g/dl at T=1 ($p < 0.05$). By T=5, lymph albumin had dropped to 1.10 ± 0.41 g/dl, but this was not significantly different from T=0. The experimental group showed a gradual decrease in lymph albumin, with values of 1.39 ± 0.22 , 1.37 ± 0.22 and 1.15 ± 0.24 g/dl at T=0, 1, and 5 respectively. These were not significantly different from control results.

Table 54 - Lymph Albumin (g/dl) for Control Animals

Pig #	Time (hrs)				
	T=0	T=1	T=2	T=3	T=5
15	0.97	1.11	1.03	1.16	1.18
17	1.21	1.24	1.32	1.29	1.50
18	0.63	0.70	0.68	0.57	0.53
20	0.97	1.18	1.29	1.11	1.07
23	1.02	1.26	1.35	1.35	1.33
26	0.81	NA	0.81	0.86	0.85
28	1.16	1.28	1.61	1.32	1.39
29	1.88	1.81	1.85	1.47	1.78
33	1.10	1.28	1.23	1.28	0.72
35	0.37	0.78	0.88	0.63	0.63
Average	1.01	1.18	1.21	1.10	1.10
Std. Dev.	0.40	0.52	0.36	0.31	0.41

Table 55 - Lymph Albumin (g/dl) for Experimental Animals

Pig #	Time (hrs)				
	T=0	T=1.0	T=2.0	T=3.0	T=5.0
13	1.20	1.07	1.23	1.21	1.23
19	1.36	1.44	1.38	1.28	0.84
22	1.10	1.16	1.49	1.35	1.31
24	1.34	1.44	1.54	1.53	1.39
27	1.37	1.32	1.31	1.29	NA
30	1.46	1.69	1.39	1.37	1.01
31	1.19	1.50	1.53	1.31	1.22
32	1.56	1.61	1.47	1.52	1.38
34	1.42	1.44	1.55	1.41	1.23
36	1.87	1.03	0.68	0.68	0.71
Average	1.39	1.37	1.36	1.30	1.15
Std. Dev.	0.22	0.22	0.26	0.24	0.24

L. Arterial Oxygen Saturation

The S_aO_2 for control (Table 56) and experimental (Table 57) animals are given. The control animals showed little variation over time, with values of 98.7 ± 1.0 , 98.9 ± 0.7 , and 98.4 ± 1.5 % at times T=1, 2, and 7. Similarly, the experimental animals showed little change, with values of 99.3 ± 0.3 , 99.4 ± 0.3 , and 99.1 ± 0.3 % at times T=1, 2 and 7. The two groups showed no significant differences between one another.

Table 56 - Arterial Oxygen Saturation (%) for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	99.9	99.9	99.9	99.9	99.9	99.9
17	97.4	97.2	97	97.9	97.8	97.0
18	99.5	99.4	98.8	98.8	98.8	99.0
20	99.2	99.1	99.0	99.1	98.9	98.9
23	99.8	99.5	99.5	99.4	99.4	99.3
26	98.8	98.9	98.9	99.0	98.8	98.9
28	99.1	99.1	99.0	99.0	99.1	99.1
29	97.7	99.3	99.4	99.2	99.2	99.4
33	99.1	98.7	99.0	99.0	98.7	98.3
35	97.4	96.3	98.5	99.2	99.2	94.5
Average	98.8	98.7	98.9	99.1	99.0	98.4
Std. Dev.	0.9	1.0	0.7	0.4	0.4	1.5

Table 57 - Arterial Oxygen Saturation (%) for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	99.9	99.9	99.9	99.9	99.9	99.9
19	98.9	98.9	99.1	98.8	99.0	98.8
22	99.3	99.2	99.2	99.2	99.0	99.0
24	99.8	99.3	99.5	99.4	99.2	97.5
27	99.2	99.3	99.3	99.1	99.4	NA
30	98.2	99.2	99.4	99.6	99.2	99.7
31	99.4	99.4	99.1	99.3	99.4	99.5
32	99.5	99.4	99.5	99.4	99.6	98.9
34	99.5	98.7	99.7	99.2	94.9	NA
36	99.1	99.1	98.8	98.9	99.1	99.2
Average	99.3	99.3	99.4	99.3	98.9	99.1
Std. Dev.	0.5	0.3	0.3	0.3	1.4	0.7

M. Venous Oxygen Saturation

The S_{vO_2} for control (Table 58) and experimental (Table 59) animals are given. Control animals showed little variation over the baseline period, with an S_{vO_2} of 69.5 ± 7.1 % at T=1, with an insignificant decrease to 67.5 ± 5.4 % at T=2. At T=7 however, there was a significant decrease to 60.9 ± 8.5 % ($p < 0.01$). The experimental animals showed little variation over the baseline period, with a value of 68.8 ± 8.1 % at T=1, and this did not differ significantly from controls. Values at T=2 and T=7, 45.2 ± 16.9 and 48.4 ± 22.2 %, did differ significantly from controls ($p < 0.001$ and $p < 0.05$ respectively).

Table 58 - Venous Oxygen Saturation (%) for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	68.2	67.7	66.5	66.4	61.1	65.8
17	76.7	72.1	69.6	66.2	59.5	50.0
18	73.3	79.8	75.3	73.7	67.4	60.2
20	67.0	66.1	71.5	64.9	58.8	61.6
23	76.1	77.1	68.0	67.7	70.1	72.8
26	67.5	68.0	64.9	62.5	60.2	59.2
28	63.0	63.7	61.5	61.9	43.8	65.7
29	71.0	72.8	72.0	73.4	69.4	68.2
33	73.0	72.9	68.9	68.6	68.9	61.6
35	57.2	54.8	56.7	55.2	52.0	43.4
Average	69.3	69.5	67.5	66.1	61.1	60.9
Std. Dev.	6.0	7.1	5.4	5.5	8.4	8.5

Table 59 - Venous Oxygen Saturation (%) for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	77.9	71.4	79.9	83.8	87.6	85.2
19	62.1	62.1	35.6	NA	NA	NA
22	72.0	71.7	53.0	47.1	52.0	NA
24	65.8	57.9	18.6	13.7	18.8	17.2
27	71.4	75.0	36.8	43.1	37.0	NA
30	42.0	64.1	55.2	48.3	62.1	48.8
31	70.2	71.5	52.8	45.3	48.5	55.5
32	64.4	67.5	37.9	41.8	35.0	40.2
34	78.2	85.8	50.8	34.7	27.3	NA
36	60.7	61.1	31.7	34.5	41.8	43.6
Average	66.5	68.8	45.2	43.6	45.6	48.4
Std. Dev.	10.5	8.1	16.9	18.4	20.4	22.2

N. Arterial Oxygen Content

The C_aO_2 for control (Table 60) and experimental (Table 61) animals are given. Controls showed a gradual increase with time, 14.9 ± 2 , 15.7 ± 2.3 , and 16.9 ± 2.2 ml O_2 /dl at times T=1, 2 and 7 respectively. Considering variation within group, only this last valued differed significantly ($p < 0.01$). In contrast, the experimental animals showed little change over time, with values of 15.7 ± 2.4 , 16.3 ± 1.9 and 14.8 ± 2.0 ml O_2 /dl at times T=1, 2 and 7. Variations within the experimental group were not significant, and only the results at T=7 differed significantly between the two groups ($p < 0.05$).

Table 60 - Arterial Oxygen Content (ml O₂/dl) for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	16.0	14.4	14.8	14.9	15.9	16.9
17	15.9	15.0	15.2	16.1	18.4	16.5
18	12.5	11.8	12.3	11.9	14.3	13.7
20	13.6	14.4	14.9	14.9	15.9	16.5
23	14.5	14.7	14.6	15.9	17.9	18.2
26	16.3	17.2	18.4	18.4	18.2	16.4
28	17.0	18.1	17.9	18.2	18.6	19.1
29	17.0	17.8	18.5	19.6	20.0	20.1
33	16.1	12.5	16.4	17.1	18.3	18.5
35	13.1	13.0	13.7	14.3	14.3	13.3
Average	15.2	14.9	15.7	16.1	17.2	16.9
Std. Dev.	1.7	2.2	2.1	2.3	2.0	2.2

Table 61 - Arterial Oxygen Content (ml O₂/dl) for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	15.4	15.9	16.5	17.1	16.3	16.1
19	11.1	11.0	12.2	11.1	11.0	10.8
22	13.0	12.9	14.3	14.0	14.6	13.7
24	17.1	16.7	16.4	15.5	16.1	15.0
27	18.7	18.0	17.9	16.1	14.2	NA
30	14.1	14.4	15.8	15.9	15.7	14.7
31	18.3	19.3	18.0	19.2	17.9	14.8
32	16.3	16.2	18.7	19.3	18.6	17.7
34	15.8	15.8	15.9	15.6	14.4	NA
36	14.8	16.4	17.5	16.5	16.6	15.5
Average	15.5	15.7	16.3	16.0	15.6	14.8
Std. Dev.	2.4	2.4	1.9	2.4	2.2	2.0

O. Venous Oxygen Content

The C_vO₂ for control (Table 62) and experimental animals (Table 63) are given. Controls showed no significant variation over time, with values of 10.1 ± 1.6 , 10.4 ± 1.5 , and 10.3 ± 2.4 ml O₂/dl for times T=1, 2 and 7. Experimental animals showed little variation over the baseline period, with a C_vO₂ of 10.6 ± 2.3 ml O₂/dl at T=1. Subsequently there was a marked decrease with values of 7.1 ± 2.6 , and 7.3 ± 3.3 ml O₂/dl at times T=2 and 7, and these were significantly different from control values ($p < 0.005$ and $p < 0.05$ respectively).

Table 62 - Venous Oxygen Content (ml O₂/dl) for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	10.2	9.0	9.1	9.1	9.0	10.5
17	12.4	11.0	10.8	10.8	11.1	8.4
18	8.9	9.2	9.2	8.7	9.5	8.1
20	9.3	9.4	10.5	9.5	9.3	10.1
23	10.6	10.2	9.6	10.6	12.4	13.0
26	10.9	11.6	11.9	11.5	10.9	9.6
28	10.6	11.4	10.9	11.2	8.1	12.5
29	12.2	12.7	13.1	14.2	13.7	13.5
33	11.6	9.0	11.2	11.6	12.6	11.4
35	7.6	7.3	7.7	7.7	7.3	6.1
Average	10.4	10.1	10.4	10.5	10.4	10.3
Std. Dev.	1.5	1.6	1.5	1.8	2.0	2.4

Table 63 - Venous Oxygen Content (ml O₂/dl) for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	11.2	10.5	12.3	13.3	13.2	12.8
19	6.8	6.7	4.3	NA	NA	NA
22	9.1	9.1	7.4	6.5	7.5	NA
24	10.8	9.5	3.0	2.6	3.0	2.6
27	13.2	13.3	6.5	6.9	5.2	NA
30	6.0	9.1	8.5	7.5	9.6	6.9
31	12.7	13.6	9.4	8.6	8.6	8.0
32	10.2	10.7	7.0	7.9	6.4	7.1
34	12.1	13.5	7.8	5.4	4.2	NA
36	8.8	9.9	5.5	5.7	6.9	6.6
Average	10.1	10.6	7.1	7.1	7.2	7.3
Std. Dev.	2.4	2.3	2.6	2.9	3.1	3.3

P. Arterial-Mixed Venous Oxygen Content Difference

The $C_{a-v}O_2$ for control (Table 64) and experimental (Table 65) animals are given. Controls showed little variation over the baseline period, with a $C_{a-v}O_2$ at T=1 of 4.8 ± 1.2 ml O₂/dl. Later results showed small but significant increases in $C_{a-v}O_2$ over time, with values of 5.3 ± 1.1 , and 6.6 ± 0.8 ml O₂/dl at times T=2 and 7 ($p < 0.05$ and $p < 0.005$ compared to T=1). Experimental animals also showed little variation during the baseline

period, with a value of 5.4 ± 1.4 ml O₂/dl at T=1 which did not differ significantly from controls. Results for experimental animals at T=2, 9.1 ± 2.9 ml O₂/dl, did differ significantly from control animals ($p < 0.001$), though those at T=7, 8.3 ± 3.2 ml O₂/dl, did not.

Table 64 - Arterial-Mixed Venous Oxygen Content Difference (ml O₂/dl) for Control Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
15	5.8	5.4	5.7	5.8	6.9	6.3
17	3.5	4.0	4.4	5.4	7.3	8.1
18	3.6	2.7	3.1	3.2	4.7	5.5
20	4.2	5.0	4.4	5.4	6.6	6.4
23	3.9	4.5	5.0	5.4	5.6	5.1
26	5.4	5.6	6.5	7.0	7.3	6.8
28	6.4	6.7	7.0	7.0	10.5	6.7
29	4.8	5.0	5.4	5.	6.2	6.6
33	4.5	3.6	5.2	5.5	5.7	7.1
35	5.5	5.7	5.9	6.5	7.0	7.2
Average	4.8	4.8	5.3	5.6	6.8	6.6
Std. Dev.	1.0	1.2	1.1	1.1	1.6	0.8

Table 65 - Arterial-Mixed Venous Oxygen Content Difference (ml O₂/dl) for Experimental Animals

Pig #	Time (hrs)					
	T=0	T=1	T=2	T=3	T=5	T=7
13	4.2	5.4	4.2	3.8	3.1	3.3
19	4.3	4.3	8.0	NA	NA	NA
22	3.8	3.8	6.8	7.5	7.1	NA
24	6.3	7.2	13.4	13.0	13.1	12.4
27	5.5	4.7	11.4	9.2	9.1	NA
30	8.2	5.3	7.3	8.5	6.1	7.9
31	5.7	5.7	8.6	10.6	9.4	6.8
32	6.	5.5	11.7	11.4	12.2	10.6
34	3.7	2.3	8.0	10.2	10.3	NA
36	5.9	6.5	11.9	10.9	9.7	8.8
Average	5.4	5.1	9.1	9.4	8.9	8.3
Std. Dev.	1.4	1.4	2.9	2.7	3.1	3.2

V. Bacteriological Results

Culture results were reported in two fashions. The first was to record organisms as identified by the microbiologist. The second was to report data in terms of positive or negative results. Results are presented for portal blood, arterial blood, lymph and lymph nodes.

Positive cultures were those in which common gut organisms were isolated, regardless of whether the culture contained other organisms considered contaminants. Negative cultures were those in which either no growth occurred after seven days, or the bacteria isolated were considered contaminants. The decision as to what constituted a common gut organism or contaminant was based on several criteria. The first was through a review of the literature regarding the normal intestinal microflora of swine^{145, 146, 147}. The second was through discussion with the microbiologist who characterized the bacteria (Dr. P. Kibsey) and an animal microbiologist (Dr. M. Schoonderwoerd, Head, Bacteriology Section, Alberta Agriculture, Animal Health Division, Edmonton, Alberta). Common intestinal microflora of the swine cultured are given (Table 66), as are the contaminants encountered (Table 67).

Table 66 - Bacteria Considered Common Enteric Organisms

<i>Enterobacter aerogenes</i> (<i>E. aerogenes</i>) <i>Escherichia coli</i> (<i>E. coli</i>) <i>Fusobacterium</i> sp. (<i>Fuso</i>) <i>Lactobacillus</i> sp. <i>Streptococcus bovis</i> (<i>S. bovis</i>) <i>Streptococcus viridans</i> (<i>S. viridans</i>)

Table 67 - Bacteria Considered Contaminants

<i>Bacillus</i> sp. <i>Hemophilus</i> sp. <i>Micrococcus</i> sp. (<i>Micro</i>) <i>Staphylococcus aureus</i> (<i>S. aureus</i>) Coagulase Negative <i>Staphylococcus</i> (CNS) β hemolytic <i>Streptococcus</i>
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A. Portal Blood

The organisms isolated from portal blood cultures are given for control (Table 68) and experimental (Table 70) animals. Positives and negatives for the groups are summarized (Table 70), and contaminants listed in parenthesis under the negative column. Control animals grew only two contaminants out of a total of forty cultures taken, with the rest showing no growth (NG). Cultures from experimental animals showed only one positive culture of thirty nine taken, with thirty-eight no growths. These results did not differ significantly for either the individual sampling periods or the overall experiment.

Table 68 - Organisms Isolated From Portal Blood of Control Animals

Pig #	Time (hrs)			
	T=0	T=1	T=3	T=5
15	NG	NG	NG	NG
17	<i>CNS</i>	NG	NG	NG
18	NG	NG	NG	NG
20	NG	NG	NG	NG
23	NG	NG	NG	NG
26	NG	NG	NG	NG
28	NG	<i>CNS</i>	NG	NG
29	NG	NG	NG	NG
33	NG	NG	NG	NG
35	NG	NG	NG	NG

Table 69 - Organisms Isolated From Portal Blood for Experimental Animals

Pig #	Time (hrs)			
	T=1	T=2	T=5	T=7
13	NG	NG	NG	NG
19	NG	NG	NG	NG
22	NG	NG	NG	NG
24	NG	NG	NG	NG
27	NG	NG	<i>Fuso</i>	NA
30	NG	NG	NG	NG
31	NG	NG	NG	NG
32	NG	NG	NG	NG
34	NG	NG	NG	NG
36	NG	NG	NG	NG

Table 70 - Summary of Portal Blood Cultures for Control and Experimental Animals

Result	Control	Experimental
Positive	0	1
Negative (Contaminant)	40 (2)	39 (0)
Total	40	40

B. Mesenteric Lymph

The organisms isolated from mesenteric lymph cultures are given for control (Table 71) and experimental (Table 72) animals. Positives and negatives for the groups are summarized (Table 73), and contaminants listed in parenthesis under the negative column. No organisms were isolated from forty cultures of control animals. Only one contaminant, *Micrococcus*, was isolated from experimental animals, and again no positive cultures were recorded. Only thirty-nine cultures were taken in this group, as one animal died prior to cultures. These differences were not significant.

Table 71 - Organisms Isolated From Mesenteric Lymph for Control Animals

Pig #	Time (hrs)			
	T=0	T=1	T=3	T=5
15	NG	NG	NG	NG
17	NG	NG	NG	NG
18	NG	NG	NG	NG
20	NG	NG	NG	NG
23	NG	NG	NG	NG
26	NG	NG	NG	NG
28	NG	NG	NG	NG
29	NG	NG	NG	NG
33	NG	NG	NG	NG
35	NG	NG	NG	NG

Table 72 - Organisms Isolated From Mesenteric Lymph for Experimental Animals

Pig #	Time (hrs)			
	T=1	T=2	T=5	T=7
13	NG	NG	NG	NG
19	NG	NG	NG	NG
22	NG	NG	NG	NG
24	NG	NG	NG	NG
27	NG	NG	Micro	NA
30	NG	NG	NG	NG
31	NG	NG	NG	NG
32	NG	NG	NG	NG
34	NG	NG	NG	NG
36	NG	NG	NG	NG

Table 73 - Summary of Mesenteric Lymph Cultures for Control and Experimental Animals

Result	Control	Experimental
Positive	0	0
Negative (Contaminant)	40 (0)	39 (1)
Total	40	39

C. Systemic Blood

The organisms isolated from systemic blood cultures are given for control (Table 74) and experimental (Table 75) animals. Positives and negatives for the groups are summarized (Table 76), and contaminants listed in parenthesis under the negative column. One of ten cultures was positive for controls, with the organism isolated being *S. bovis*. Of the nine negative cultures, eight showed no growth, and one grew a contaminant, Coagulase Negative *Staphylococcus*. Experimental animals showed one positive result from ten cultures, with *S. viridans* being isolated. Eight of nine negative results for experimentals showed no growth, with one specimen growing Coagulase Negative *Staphylococcus*. These results did not differ significantly.

Table 74 - Organisms Isolated From Systemic Blood for Control Animals

Pig #	Organism
15	CNS
17	NG
18	NG
20	NG
23	NG
26	NG
28	NG
29	<i>S. bovis</i>
33	NG
35	NG

Table 75 - Organisms Isolated From Systemic Blood for Experimental Animals

Pig #	Organism
13	CNS
19	NG
22	NG
24	<i>S. viridans</i>
27	NG
30	NG
31	NG
32	NG
34	NG
36	NG

Table 76 - Summary of Systemic Blood Cultures for Control and Experimental Animals

Result	Control	Experimental
Positive	1	1
Negative (Contaminant)	9 (1)	9 (1)
Total	10	10

D. Mesenteric Lymph Nodes

The organisms isolated from mesenteric lymph node cultures are given for control (Table 77) and experimental (Table 78) animals. Positives and negatives for the groups are summarized (Table 79), and contaminants listed in parenthesis under the negative column.

Control animals showed three of ten cultures positive, with *E. aerogenes*, *Lactobacillus*, and *S. bovis* being isolated. Of the seven negatives in this group, six did grow contaminants. The experimental group showed four of ten cultures positive, with *E. coli*, and *S. bovis* being isolated. Of six negatives in this group, five were contaminants. There was no difference between the two groups.

Table 77 - Organisms Isolated From Mesenteric Lymph Nodes for Control Animals

Pig #	Organism
15	<i>E. aerogenes</i>
17	<i>S. aureus</i> , CNS
18	CNS, <i>Lactobacillus</i>
20	<i>S. aureus</i> , β hemolytic <i>Strep.</i>
23	CNS
26	<i>Bacillus</i>
28	<i>S. bovis</i>
29	NG
33	<i>Bacillus</i>
35	<i>S. aureus</i>

Table 78 - Organisms Isolated From Mesenteric Lymph Nodes for Experimental Animals

Pig #	Organism
13	<i>E. coli</i> , CNS, <i>S. aureus</i> , β hemolytic <i>Strep.</i>
19	CNS
22	<i>S. viridans</i> , CNS
24	<i>S. aureus</i>
27	<i>S. viridans</i>
30	CNS, β hemolytic <i>Strep.</i>
31	<i>S. aureus</i> , <i>E. coli</i>
32	NG
34	<i>S. aureus</i>
36	<i>Hemophilus</i>

Table 79 - Summary of Mesenteric Lymph Node Cultures for Control and Experimental Animals

Result	Control	Experimental
Positive	3	4
Negative (Contaminant)	7 (6)	6 (5)

VI. Bowel Histology

Samples of terminal ileum were obtained from all animals, and examined microscopically. All samples were graded from zero to three according to the degree of inflammation present (Table 80). Results for control and experimental animals are given (Table 81). These differences were not significant.

Table 80 - Histological Grading Scale

Grade	Findings
0	No lesion
1	Focal Peritonitis
2	Microabscesses, Diffuse Peritonitis
3	2 With Extension Into Muscularis

Table 81 - Histological Grading of Inflammation for Control and Experimental Animals

Control Pig	Grade	Experimental Pig	Grade
15	3	13	0
17	1	19	3
18	0	22	0
20	0	24	0
23	3	27	0
26	1	30	3
28	0	31	0
29	2	32	3
33	0	34	0
35	3	36	0
Total	13	Total	9

DISCUSSION

Bacterial translocation has been promoted as an explanation for sepsis commonly associated with hemorrhagic shock. Most studies have used small animal models and thus their clinical application is questionable. The major objective of this study was to determine if bacterial translocation occurs in a large animal shock model. A second objective was to determine possible mechanisms involved. Results of this study will be considered, but before doing so the experimental design will be examined.

I. Experimental Design

In studying human disease, models are an attempt to reproduce the situation found in man as closely as possible. The model presented has advantages over other models discussed in the literature, but at the same time, it has drawbacks which should be addressed.

To date, no study has attempted to demonstrate bacterial translocation associated with hemorrhagic shock in a large animal model. For the most part, various strains of rodents have been used in such work. The reasons for this are many, but certainly cost, ease of handling, and familiarity are factors. In addition, the immune system of these animals has been extensively studied. There are however several disadvantages to using rodents, and these have already been discussed (*vide supra*). The domestic swine resembles the situation found in man in many instances, and has become a valuable research model¹⁴⁸. Gastrointestinal¹³² and cardiovascular¹³³ physiology are felt to closely parallel that of man, and thus the swine seems well suited to study bacterial translocation and shock.

A related issue is the specific source of animals for this study. Domestic swine are known to have a number of endemic diseases which might affect response to stress. In preliminary experiments, minimally diseased animals were used; however, these pigs demonstrated malignant hyperthermia, a trait common to swine¹⁴⁹. For this reason a new

source of animals was obtained for the formal experiments. These new swine were all obtained from one supplier, but did not appear as healthy as the minimally diseased pigs. Unfortunately, limitations of space and funding did not allow for isolation and observation of the animals prior to surgery in order to ensure well being.

In the clinical setting, the patient is rarely anesthetized during hemorrhage, and current trends in shock research have been to use conscious animals with chronic indwelling catheters^{150,134}. This was not done in this experiment for several reasons. First, it is unlikely that the catheters would have remained in position in an awake animal. Second, the resources were not available to perform such a study. Third, most chronic experiments of this type give animals prophylactic antibiotics to prevent infection, something which would be unacceptable in an experiment examining bacterial translocation.

Given that the experiment was done acutely, anesthesia was necessary. The choice of anesthetics was made after reviewing the literature¹⁴⁹, discussing the possibilities with other investigators¹³⁵, and determining what was available within budget constraints. Ketamine and atropine were chosen for premedication as they allow easy induction, minimize secretions, and are short acting¹⁵¹. Halothane was used as the initial agent as it provides excellent anaesthesia, and is readily available. Its disadvantages are cardiac depression and loss of splanchnic bed autoregulation¹⁵², and for these reasons was not used for the entire experiment. Rather, halothane was only used during the operation, and at low concentrations to minimize effects during the experimental period. Sodium pentobarbital provides excellent sedation, though it has limited action as an analgesic¹⁵³. It too may depress cardiac function, though less than halothane¹⁵², and can decrease vascular tone. Overall, the combination of agents was chosen to provide adequate analgesia and sedation, while minimizing cardiovascular side effects.

Once anesthetics were used, the question of ventilation arose. It was decided that in order to provide adequate depth of anaesthesia, assisted ventilation was necessary. In

addition, it was felt that variations with spontaneous respiration would confuse hemodynamic monitoring. Inspired oxygen and P_{aO_2} were kept high to mimic the clinical situation, as patients in shock usually receive oxygen therapy.

Hemorrhagic shock models are usually bled in one of two ways. The first, known as a Wiggers preparation¹⁵⁴, involves bleeding the animal into a reservoir until a desired pressure is obtained. This is maintained for a set period of time by either removing or returning blood to the animal. The second, more clinically pertinent, method was used in this experiment, and involves bleeding the animal a predetermined percentage of the estimated blood volume, in this instance 40%. This percentage was chosen to cause significant shock, as determined by hemodynamic and biochemical parameters, without causing rapid death. That three of ten experimental animals died within six hours indicates that the insult was severe. Several attempts were made to bleed animals 50% of blood volume, however these were uniformly fatal within one hour. Most studies in translocation and shock have utilized the less popular and less relevant Wiggers model¹⁵⁵. Furthermore, these studies have involved extremely prolonged and severe forms of shock, with animals being maintained at pressures of 30 mm Hg for several hours. Such insults in humans would be almost uniformly fatal.

Lack of resuscitation in this model is certainly one area of possible criticism. The usual clinical scenario is to have the patient resuscitated shortly after the insult. This was omitted for several reasons. Both time and resources were limited, and it would not have been possible to include a third group of resuscitated animals. Furthermore, as it is not clear whether translocation is an ischemic or reperfusion phenomenon, it was decided to test the premise that ischemia is the important event. This laboratory plans to continue the study with resuscitated animals in the future.

Length of time the animals were followed posthemorrhage is also debatable. In this study animals were observed for six hours after bleeding. Other investigators have had followup periods of up to 72 hours. However, studies documenting translocation have

usually been able to do so within 5 hours of the initial insult^{122,123}, and so 6 hours seemed a reasonable period of time. Furthermore, because of the long preparation time involved, extending the observation period would have required considerably greater resources.

The bacteriological component of this study raises several points for consideration. If the gastrointestinal tract is the source of sepsis associated with shock, cultures of portal blood, mesenteric lymph and lymph nodes should document this. Most studies have documented translocation by mesenteric lymph node culture⁴, though others have examined liver¹¹⁹, systemic blood, and to a limited degree portal blood¹²². This is the first study where mesenteric lymph and lymph nodes, systemic and portal blood have been sampled.

Ideally, the animals intestinal microflora at the beginning and end of the experiment should have been documented. This may have permitted more accurate assignment of culture results to positive and negative groups, and ensured animals did not have unusual intestinal microflora which might affect translocation. Stool cultures were not taken because of the cost involved. Subsequently, the services of a veterinarian microbiologist became available, and stool samples prior to surgery and at completion of the experiment were obtained for the last two animals, one control and one experimental. In both cases, the preoperative intestinal flora correlated well with that documented in the literature, but the postoperative cultures showed an overgrowth of *E. coli*. As mentioned in the literature review, one method to promote translocation is to monoassociate the gut with one strain of bacteria, and *E. coli* is one of the most common bacteria used. However, neither of the last 2 animals tested positive for *E. coli* translocation.

Retrospective assignment of cultures to positive and negative groups was less desirable than having prospective criteria. Because native intestinal flora was used, this was not possible. This decision might result in considerable bias, however, this was not shown to be a significant factor.

Sampling methods for culture deserve comment. While portal and systemic blood was drawn and placed immediately in aerobic and anaerobic media, lymph was allowed to

remain in an oxygenated environment prior to culturing. This may have interfered with anaerobic cultures. While samples could have been collected in an anaerobic chamber, the tubing used was permeable to oxygen. Most studies have not identified anaerobes as common organisms to translocate, and this was not felt to be a major weakness of this study. Lymph nodes were cultured several hours after retrieval because of the time at which the samples were delivered to the laboratory. This could have been corrected by grinding and plating the specimens at laparotomy, had resources and personnel been available for this. Mesenteric lymph nodes had a high contamination rate which may have been due to an error in sampling technique. In most studies on translocation, laparotomy was done immediately prior to node retrieval. In this study an initial laparotomy was performed to allow cannulation of the portal vein and mesenteric lymphatics, and a second laparotomy done after the experimental period to retrieve nodes. This likely increased environmental contamination. Furthermore, because limited assistance and equipment was available at the time of the second laparotomy, it was very difficult to ensure sterile technique and to excise the peritoneum overlying the mesenteric lymph nodes.

These are some of the major strengths and weaknesses which are recognized with this model as described. With these in mind, results will now be discussed.

II. Preoperative Status

Both groups were similar with regards to preoperative weight, volume of intravenous fluids received and urine output prior to hemorrhage, and hemoglobin. Furthermore, neither group differed significantly for any hemodynamic or biochemical parameters measured during the baseline period. Baseline data for the group as a whole was similar to values reported the literature¹⁴⁰. However, such comparisons should not be overemphasized, as most studies involved conscious animals.

III. Hemodynamic Results

Hemodynamic monitoring was done in order to document the degree of shock, and to ensure that the two groups remained comparable for variables such as respiratory rate and intravenous fluids infused.

The common clinical parameters used in following shock include heart rate, mean arterial pressure and urine output. In this study experimental animals showed large and significant changes in these parameters when compared to controls. The experimental group developed marked tachycardia and profound hypotension after hemorrhage, as would be expected. Urine output in experimental animals had significantly higher urine output prior to bleeding. The reasons for this are unclear, but even so, they demonstrated a significant decrease following hemorrhage.

Swan Ganz catheter measurements further documented the degree of shock. Right atrial pressure decreased in the experimental group indicating a significant decrease in central venous return. Decreased PWP in experimental animals reflected decreased left atrial filling. Significant drops occurred in RAP and PWP shortly after hemorrhage (at T=2 hr) but by T=7 hr these decreases were no longer significant. This likely represented recovery secondary to capillary refill. The experimental animals also showed a marked decrease in CI shortly after hemorrhage. Again at T=7 hr, CI was not different from the control groups, suggesting recovery. Control animals also demonstrated a significant decrease in CI, and it seems reasonable that this represents the cumulative effect of the anesthetic agents and other stresses.

Craniomesenteric arterial flow is important if one recalls the questions raised regarding splanchnic flow in dogs and rats (*vide supra*). Initially, the experimental pigs showed a significant decrease in CAF, but by T=7 hr, these values had returned to control levels. This is more in keeping with the situation found in man and other animals⁵⁹. Unfortunately, portal venous pressures were not recorded, as it was felt that the constant

infusion required to maintain such monitoring would interfere with collection of sterile portal vein samples.

Several points are worth mentioning regarding temperature. The first is that central core temperatures are not routinely measured in swine, and thus it is not possible to compare this data with other studies. Second, it would seem that controls were slightly hypothermic following surgery, in spite of efforts to prevent this. Third, experimental animals did show a significant increase in temperature with time. The exact cause of this is not clear. It would be tempting to ascribe this to sepsis, but as will be discussed, this is not supported by culture results.

Lymph flow in experimental animals increased over time when compared with controls, however these differences were not significant. This does not concur with work in sheep⁴², and may be due to differences in experimental setup.

In summary, the hemodynamic data clearly documents that experimental animals underwent significant shock when compared to control animals. Clinical parameters usually monitored in hemorrhaged patients, such as heart rate, mean arterial pressure and urine output, showed characteristic changes of hemorrhagic shock in the experimental group. Swan Ganz catheter measurements in experimental animals supported this. Blood flow to the gastrointestinal tract was also severely reduced in the experimental group when compared to the controls.

IV. Biochemical Results

As with hemodynamic data, biochemical measurements were used to document the degree of shock and to ensure both groups remained comparable for certain variables such as PO_2 . Parameters such as $[H^+]$, Hg , P_vO_2 and $C_{a-v}O_2$ showed significant changes in experimental animals when compared to controls. Experimental animals became acidotic shortly after hemorrhage, though they returned to normal by the end of the experiment. This may have been due to changes in respiratory rates. Not surprisingly, HCO_3 decreased

for experimental animals at the same time. Hemoglobin for control animals showed a gradual, but not significant rise over the course of the experiment. Unfortunately preoperative *Hg*s were not drawn. The experimental group showed a significant drop in *Hg* at $T=7$ hr only, reinforcing that *Hg* is often a poor indicator of acute blood loss. Hematocrits showed similar changes.

The two groups showed no significant differences for P_aCO_2 or P_aO_2 , indicating that ventilation and oxygenation were comparable. Mixed venous oxygen dropped significantly in the experimental group at $T=2$ hr, reflecting increased oxygen extraction secondary to decreased oxygen delivery. Arterial and venous oxygen saturations and contents were used to calculate $C_{a-v}O_2$. Control animals demonstrated an increase in $C_{a-v}O_2$ with time, likely due to the stress of the experiment. Experimental animals showed an even greater increase in $C_{a-v}O_2$, and differed significantly from controls at $T=2$ hr. This represented the same changes discussed for P_vO_2 .

Total protein and albumin for serum and lymph were measured. Lymphatics are almost unique in their ability to return protein from interstitium to the vascular system³³, and thus changes in their permeability in shock would be of interest. Furthermore, with regards to translocation, increased permeability might apply for bacteria as well. Unfortunately these measurements would be a crude guide at best to such changes, and even more so because of the manner in which they were measured. It would have been ideal to utilize electrophoresis, but this would have entailed an enormous expenditure of time and money. In general, serum total protein and albumin tended to decrease in the experimental group after hemorrhage, however these differences were not significant. Lymph total protein and albumin also tended to decrease in the experimental animals, but again these differences were not significant. Preliminary calculations were made to determine actual quantities of protein and albumin in lymph, by multiplying concentration by volume. These did not reveal any significant differences between groups. Thus, there appears to have been no change in the permeability of the circulatory system. This raises

the issue of whether the insult in this study was adequate to activate the inflammatory cascade. Results for total protein and albumin suggests this was not the case, as no change in systemic permeability was documented. This cannot be stated categorically.

In summary, biochemical data further documents that experimental animals underwent significant shock when compared to controls. Increased $[H^+]$, decreased Hg , Hct , P_vO_2 and especially $C_{a-v}O_2$ in the experimental group supports this conclusion.

V. Bacteriological Results

In assessing the bacteriological component of this study, the first question raised is whether the model was free of contamination. Only four of ninety aerobic and anaerobic cultures, excluding lymph nodes, showed any growth after seven days for control animals. Of these four, three were contaminants, for a contamination rate of 3.3%. Experimental animals had a similarly low rate, with only two contaminants in eighty-nine cultures. The results for lymph node cultures were less impressive, with both groups having high rates of contamination. This may represent difficulty in obtaining sterile samples of lymph nodes when performing a second laparotomy without adequate assistance and equipment. This problem was recognized and attempts made to correct the situation. In later experiments greater care was taken in retrieving nodes, and this appeared to improve matters. Contamination rates were lower in subsequent animals, with fewer contaminating organisms per animal. Perhaps more importantly, nodes from two of the later animals showed no growth.

The second question was whether the culture methods used were sufficiently sensitive to detect bacteria. With regards to lymph and blood culture techniques, the system used is one of the most sensitive available. It is routinely tested, and has been able to detect as little as one colony introduced into culture bottles¹⁵⁶. The technique used for culturing lymph nodes is a standard one, and is also routinely tested for sensitivity. That

both systems are used in the clinical setting is further support that they are adequate for this experiment. Discussion with the veterinarian microbiologist supported these points.

The final question is whether there is a difference in culture results between controls and experimentals. Results of cultures of portal and systemic blood, mesenteric lymph and lymph nodes showed no significant difference. It might be argued that the method for assigning positive and negative cultures resulted in confounding bias, however calculations were made treating any growth as positive, and again no differences were demonstrated. It is of interest that one portal blood culture from an experimental animal grew *Fusobacterium*, an anaerobic organism. Review of the literature shows that most studies have not documented anaerobes translocating, and one study has suggested anaerobes may inhibit translocation¹⁰³.

VI. Histological Results

Portions of the terminal ileum were examined histologically to determine if there were any differences between the two groups. No significant differences were found. The changes observed were quite nonspecific, and seen in a minority of the animals. Their etiology may have been due to problems in sampling technique, or trauma to the bowel during laparotomy.

CONCLUSIONS

Hemorrhagic shock is a common cause of morbidity and mortality. Sepsis appears to be a major factor in this problem. In the clinical setting, when a source of infection is identified, steps can be taken to correct it. Furthermore, identifying such sources permits development of strategies which prevent infections from occurring and thus reducing morbidity and mortality. When no source is identified prophylaxis is not possible, and treatment more difficult. Numerous investigators over several decades have attempted to implicate the gut as a major source of sepsis associated with hemorrhagic shock. If this is the case, steps might be taken to address this problem, both for prophylaxis and treatment. One need only recall the effect of mechanical cleanout and antibiotic prophylaxis on infection rates associated with colon surgery to appreciate the possible impact of such research. The current theory to explain the apparent relationship between shock, sepsis and the gut is bacterial translocation. Human studies towards proving this theory are limited by ethical considerations, and thus animal models must be relied on. Most models to date have involved rodents, and are less than ideal.

The main objective of this study was to answer the question, can bacterial translocation be demonstrated in a large animal model of hemorrhagic shock? With certain qualifications, the answer is no. Specifically, in an unresuscitated swine model, the data presented fails to demonstrate increased bacterial translocation within 6 hours of hemorrhage. This suggests that ischemia alone is not sufficient to increase bacterial translocation in this model.

This information raises several interesting possibilities with regards to sepsis associated with hemorrhagic shock. The first is that bacterial translocation is not increased within 6 hours of hemorrhage and that the gut is not the source of sepsis in this setting. This is a very real possibility, though it contradicts much of the current literature. Studies to date have largely been confined to animal models which do not mimic the situation in man. Furthermore, the only human study was unimpressive in terms of the number of

true enteric organisms cultured⁹⁰. The fact that increased translocation was not demonstrated in a swine shock model supports this. However, the issue is hardly put to rest. The work done in rodents has been impressive, and while these models have major drawbacks, several independent investigators have been able to produce results which support the role of translocation as a source of sepsis.

The second possibility is that translocation is a reperfusion injury and that without resuscitation it may not occur. This too is a credible suggestion, and this model did not feature a resuscitated group. Nevertheless, experimental animals did show a certain amount of recovery and thus some degree of reperfusion likely occurred.

Other possibilities may be that the degree of hemorrhage was inadequate to produce translocation, or that the followup period was insufficient. Forty percent hemorrhage in a human is an extremely serious insult, but it may be true that the degree of shock in this model was inadequate to produce translocation. If this is the case however, the clinical importance of this phenomenon is questionable. Patients shocked to this degree generally have an extremely high mortality, regardless of sepsis. The length of followup has already been discussed, and while most rodent studies demonstrated translocation within five hours of insult, a longer followup after hemorrhage should be considered.

RECOMMENDATIONS

As long as hemorrhagic shock remains a major cause of morbidity and mortality, it warrants further study. Because sepsis is a major factor in determining outcome in this setting, research into identifying sources of infection in shocked patients offers great promise. If the gastrointestinal tract, through bacterial translocation, is a major source of sepsis associated with hemorrhagic shock, then demonstrating this may result in improved treatment and prophylaxis. For these reasons, the following recommendations are offered:

1. Research should continue in the area of bacterial translocation and hemorrhagic shock, with the goal of ascertaining whether this phenomenon can be demonstrated in a large animal model which approximates the human situation. Given the physiological similarities between man and swine, the model developed in this thesis seems appropriate. Areas for improvement in this model have been identified and include:
 - a. A reliable source of healthy swine and the facilities to observe animals prior to surgery must be secured.
 - b. Consideration should be given to a chronic experiment with indwelling catheters and without the use of anesthetics during hemorrhage.
 - c. This study should be continued with the use of a group of hemorrhaged animals who are fully resuscitated.
 - d. Animals should be followed for a longer time after hemorrhage, possibly 12 or 24 hours.
 - e. Stool cultures should be obtained before and after the experiment.
 - f. Culture techniques should be refined. Specifically, the way in which lymph nodes are retrieved and processed requires improvement.

2. Given the paucity of evidence of the gut as a source of sepsis with shock in human studies, efforts should be continued towards documenting this phenomenon in hemorrhagic shock patients.
3. Should these two recommendations prove successful, consideration should be given towards strategies which would reduce the bacterial content of the gut in hemorrhagic shock patients. These could include mechanical and antimicrobial therapy.

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